Queensland **the Smart State**

Inland Prawn Farming

Studies into the Potential for Inland Marine Prawn Farming in Queensland

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ABBREVIATIONS, UNITS AND ACRONYMS

ADSA	Australian Dryland Salinity Assessment
ANZECC	Australian and New Zealand Environment Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
B	Burdekin
BIARC	Bribie Island Aquaculture Research Centre
Ca^{2+}	Calcium
$CaSo_4$	Calcium Sulphate
Cl^-	Chloride
CW	Central West
cm	centimeter
DD	Darling Downs
DO	Dissolved Oxygen
DSSC	Desert Sweet Shrimp Company
DSW	Diluted Seawater
DNRM	Department of Natural Resources and Mines
DNR	Department of Natural Resources
EC	Electrical Conductivity
FCR	Food Conversion Ratio
GAV	Gill Associated Virus
ha	Hectare
hp	Horsepower
IPF	Inland Prawn Farming
K^+	Potassium
kg	Kilogram
kw	Kilowatt
L	Litre
LV	Lockyer Valley
NLWRA	National Land and Water Resources Audit
Mg^{2+}	Magnesium
mg	Milligrams
ML	Megalitre
mm	millimeter
Na^+	Sodium
NW	North West
P	Pond

PL	Postlarvae
ppt	parts per thousand
QMDB	Queensland Murray-Darling Basin
RSB	Relative Seawater Balance
µm	micrometers (10^{-6})
µS	micro siemens
SB	Surat Basin
SE	South East
SPF	Specific Pathogen Free
SO_4^-	Sulphate
SW	South West
SWC	Seawater Control
t	Tonne
TDI	Total Dissolved Ions
TGW	Treated Groundwater
UGW	Untreated Groundwater
USA	United States of America
WSSV	White Spot Syndrome Virus
YHV	Yellow Head Virus

SUMMARY

This project was undertaken to investigate the potential for marine prawn farming in inland regions of Queensland. With a reputation for high quality produce and strong local demand, prawn farming is one of Australia's most successful aquaculture industries. Inland prawn farming (IPF) has been successfully practiced in many countries including the United States, Thailand, China, Ecuador and India. These countries provide ready examples of how IPF can be successfully implemented in Queensland. The driving forces behind inland production have been the prevention of disease, increasingly stringent effluent conditions for coastal farms and opportunity for low cost production. Successful implementation of IPF in Queensland will be dependent on the availability and suitability of groundwater, appropriate environmental regulation and the overall profitability of the activity. This study identified the types of water suitable for IPF, regions with potential for development, the types of systems that would need to be developed and the likely performance of those systems. The results of this work demonstrated that the use of Queensland's groundwater resources could be carefully managed to provide regional communities with a sustainable and productive new industry opportunity.

1. Introduction

The Australian Dryland Salinity Assessment (ADSA, 2000) identified that the amount of land affected by salinity in Australia was 5,658,000ha - even without considering Queensland. By 2050 it is estimated that this figure will rise to at least 17,000,000ha. It has been estimated that in 2001 some 48,000ha of land was seriously affected by induced salinity (Gordon, 2002). However, up to 3,100,000ha of land in Queensland is rated as a high salinity threat and could be affected by salinity in 2050 (ADSA, 2000). If Australia is to maintain its strong farming traditions, natural resource management practices must work within the context of Australian soils, water resources and climate (ADSA, 2000).

Rising groundwater levels in many regions are forcing farmers to either abandon formerly productive areas or introduce salt-tolerant crops and farming practices. The need to develop new farming systems that reflect the balance of water quality and quantity in the landscape, do not contribute to further degradation but are productive and profitable, has sparked interest in the use of inland saline water for aquaculture. In 1997 the first national inland saline aquaculture workshop was convened in Perth (Smith and Barlow, 1997). At this workshop it was recognised that along with marine finfish and salt tolerant freshwater fish, marine crustaceans represented a significant opportunity for inland saline aquaculture. However, not all saline aquifers are likely to be suitable for prawn farming due to the strong influence of local geology on the chemistry of these waters. In addition to the quality, the supply of sufficient quantities of groundwater and its disposal of saline effluent are potential constraints to the development of inland prawn farming.

In Queensland 1,400,000 ML of groundwater are used annually with the biggest user being irrigated agriculture. A great proportion of this water contains levels of salt that, while still suitable for irrigation, might also make it suitable for the production of marine prawns. Black tiger prawns (*Penaeus monodon*), grow well at a range of salinities from full strength seawater to less than 0.5ppt salt (Pantastico and Oliveros, 1980; Saha et al., 1999). In aquaculture, water with a salinity of less than 1ppt is classed as freshwater (Boyd, 2002). The ability to grow marine prawns successfully across a range of salinities, from fresh to full strength seawater makes this a potentially significant prospect for inland aquaculture in Queensland.

Presently the majority of prawn farms in Australia are located in Queensland although they also exist in northern New South Wales, the Northern Territory and Western Australia. The value of Queensland's marine prawn production has fluctuated in recent years but peaked in 2001/02 at AU$60 million dollars (Lobegeiger and Wingfield, 2004). The three main species of prawns farmed in Queensland are the black tiger prawn (*P. monodon*), banana prawn (*Penaeus (Farfantepenaeus) merguiensis*) and Kuruma prawn (*Penaeus (Marsupenaeus) japonicus*). Situated close to accessible marine waters, these operations are typically flow-through operations which release effluent water back into coastal waterways. These farms are regulated by the Environmental Protection Agency in accordance with the amount and concentration of nutrient they release. Increasing but varying degrees of water treatment and re-use has seen a number of operators decrease their nutrient discharge in recent years. Although attempts have been made to produce prawns extensively in Australia, the desire to obtain higher levels of productivity has seen most farms develop as semi-intensive operations. In Queensland in 2003/04 a total of 2,861t of prawns were produced from 837ha at an industry average of just over 3.4t/ha (Lobegeiger and Wingfield, 2004).

There are many established examples of inland prawn farming in other countries including Thailand, Brazil, China, Ecuador, India, Israel, Mexico, Peru, and the United

States (Boyd, 2002). These examples range from farms that utilise saline groundwater as their water source through to farms that raise the salinity of freshwater ponds via the introduction of marine brine. In both cases, the effluent water is either recycled for use on subsequent aquaculture crops, disposed into the environment, or used to irrigate terrestrial crops. Those farms that utilise groundwater are often required to supplement their water with mineral fertilisers to address ionic imbalances that affect prawn growth and survival (Boyd, 2001a; Saoud et al., 2002; Boyd, 2003; McGraw and Scarpa, 2003). Commercial inland prawn farming therefore tends to be conducted at low salinities due to the cost of brine and mineral additions. A recent study of inland prawn farms in China, Thailand, Ecuador and the United States found that 68% of farms used water that was below 5ppt (Boyd and Thunjai, 2003). The two species of prawn that are presently farmed commercially at low salinity are the black tiger prawn (*P. monodon*) and the Pacific White shrimp (*Litopenaeus vannamei*).

As with coastal farms, global examples of inland prawn farming range from extensive low intensity systems through to extremely intensive systems.

1.1 Thailand

In 1998, an estimated 40% of Thailand's entire prawn crop was harvested from inland regions traditionally known for rice growing rather than aquaculture (Limsuwan, 1998). Prawn farms rapidly expanded in inland areas using low salinity culture technologies, which had initially been developed to overcome seasonal salinity fluctuations in coastal areas. At its peak in the mid to late 1990's, these inland prawn farms, which were usually less than 1 ha in size, covered a combined area of more than 22,000 ha (Szuster and Flaherty, 2000).

While highly successful, this form of inland production relies on importing brine from coastal saltpans or brine farms (Fast and Menasveta, 2000). This brine is used to acclimate prawn postlarvae, typically in bunded areas within the pond, to progressively lower salinities (Figure 1.1). By the end of the production cycle, salinities can reach as low as 0.1ppt. Water from these ponds is released into adjacent irrigation canals or freshwater habitats during the course of production or at harvest.

The potential for damage to productive rice growing regions through salinisation resulted in a ban on inland *P. monodon* production in 1998 (Fegan, 2001). Rice production is worth billions of dollars a year to the Thai economy but it also has significant cultural heritage value. Yet despite the ban, inland prawn farming continues in the central plains regions (Figure 1.2). However, a recent field survey of inland farms indicated that while no new farms were being developed, earthworks on existing farms continued (Szuster and Flaherty, 2002). Media reports indicate that in the central Nakhon Pathom region up to 6,000 farms continue to operate over 6500ha of prawn ponds in 2003 (Samabuddhi, 2003). In the last few years the culture of the white shrimp *L. vannamei* has gained in popularity in Asia with production rivalling *P. monodon* in 2003 (Merican, 2004). *L. vannamei* grows well at extremely low salinities on lower protein feeds making them an excellent species for inland production. Since the ban on inland prawn farming in freshwater areas in Thailand is specific for *P. monodon*, inland farms have rapidly taken up *L. vannamei.* Some farms have recently commenced the development of inland *L. vannamei* hatcheries (Disyabut, 2004).

The Thai approach to inland prawn farms is not suitable for Australia. The practice of importing brine from the coast to increase the salinity of freshwater is likely to result in the degradation of soil and water quality. Also the scale of the majority of inland Thai farms is too small (eg. 6,000 farms on 6,500ha in Nakhon Pathom). Australian farms would need to achieve much greater economies of scale in order to be commercially viable. Currently in Queensland there are 37 producing farms comprising some 800ha of production ponds (Lobegeiger and Winfield, 2004).

1.2 United States of America

Inland production of marine prawn in the USA has taken many forms but typically utilises groundwater to produce the Mexican white shrimp *L. vannamei*. The first attempts at inland prawn farming were undertaken almost thirty years ago by a group of farmers in west Texas (Treece, 2002). Although not commercially successful, these efforts were followed by research that was commercialised in the same region almost twenty years later. Inland prawn culture is now practiced in a number of other states including Arizona, Alabama, Mississippi, South Carolina and Florida. These farms vary from low intensity extensive through to highly intensive operations. Unlike farms in Thailand, pond farmers in the USA do not introduce marine brines to increase salinity but rely on the use of salt affected aquifers with suitable water chemistries. Most farms therefore operate at low salinities (<5ppt).

Presently the largest inland producer of prawn in the USA is OceanBoy Farms Inc. Located in central Florida, this company raises *L. vannamei* intensively using fully lined, recirculated ponds, according to strict environmental and biosecurity protocols. Low salinity groundwater (1.6ppt) is pumped from a series of bores directly into fully lined production ponds (Figure 1.3). No water is exchanged during the production cycle. Any water discharged during harvest is collected and treated in constructed wetlands before being returned to the production ponds for use on subsequent crops (Figure 1.4). The farm produces up to 24t/ha/yr, which represents an average of 12t/ha/crop from two crops per year. This product is processed at the company's facilities and sold directly to a large supermarket chain, other wholesalers and retail outlets. These products can be supplied as either green or cooked, and value added fresh and frozen prawn. The product has been recently certified organic by the United States Department of Agriculture.

In Arizona there are four prawn farms in the Sonara Desert. All of these are open pond farms but the layout and management of each of these farms are quite different. Production in Arizona peaked in 2002 at 240t but dropped in 2003 to 140t because of poor prices and an unwillingness to stock heavily because of concerns over market prices. Production had been steadily increasing since inland farming first started in 1998. In 2001 Desert Sweet Shrimp Company (DSSC) near Gila Bend produced 125t of prawn using a combination of indoor nursery systems and outdoor growout ponds (McIntosh et al., 2003). Production from this farm was scaled back to 80t in 2002 and to 55t in 2003 due to a decline in wholesale prawn prices. Despite the reduced production in 2003, the farm's productivity was the best to date with 7.6t/ha of prawn produced. At 1.5ppt, the groundwater used at DSSC is low but this allows the water to be used to irrigate salt tolerant crops such as olive trees (McIntosh et al., 2003). DSSC markets directly to consumers in nearby Phoenix through its own DSSC cafés and also through farm gate sales, its own online store, and local grocery outlets.

Other farms in areas commonly associated with catfish farming, such as Alabama and Mississippi, are also using low salinity groundwater, on a low to zero-exchange basis, to produce yields of between 4 and 6t/ha/crop. Green Prairie AquaFarm P/L, Gulf Inland BayBoy Farm P/L are two examples of inland prawn farms that have developed recently in Alabama using saline groundwater. Farms like these have a production window of around 120days and produce only one crop per year.

Figure 1.1 OceanBoy Farms Inc is located in central Florida and uses intensive open pond recirculation technologies to raise white shrimp (*L. vannamei*) at salinities less than 2ppt. (Source; www.oceanboyfarms.com)

Figure 1.2 Mangrove seedling ponds at OceanBoy Farms Inc., Florida USA. (Source; www.oceanboyfarms.com)

Another style of farm being developed in the USA are indoor recirculation facilities. A number of commercial variations of these systems are being developed to supply fresh prawn on a year round basis. One company, Sable Bay P/L at Vero Beach in Florida, uses low salinity groundwater to produce *L. vannemei* in intensively stocked raceways (up to 150 animals/m^2) that are enclosed in a tunnel constructed from a polyethylene cover. Research continues into the efficacy and efficiency of these facilities, especially with respect to the cost of production compared to pond based systems.

1.3 Ecuador

Several inland prawn farms have developed in Ecuador as recently as 1999. Many have been established on defunct redclaw crayfish operations (Salame and Salame, 2002). In 2001 coastal prawn farms were devastated by disease resulting in a significant fall in production. While these inland farms had the advantage of initially being disease free, namely of White Spot Syndrome Virus (WSSV), poor biosecurity protocols have resulted in the introduction of contaminated stock or brine into many of these facilities. Outbreaks of WSSV in inland farms were reported recently (Alava, 2004). Despite these problems, early trials returned yields of between 4 and 5 t/ha/crop. As many of these farms are in areas subject to high levels of rainfall, the farmers typically add mineral salts through the course of the crop to prevent salinity falling below productive levels (Salame and Salame, 2002).

At present almost 600ha of inland prawn farms are located in Guayas province of Ecuador (Jory, 2004). These farms all utilise low salinity groundwater. Mexico and other South American countries (eg. Brazil, Panama, Peru and Venezuela) are also rapidly developing inland prawn farming like Ecuador.

Environmental impact assessment of inland prawn farms in Ecuador is obligatory for any inland prawn farming development. This has developed because of concern over potential for salinisation of freshwater aquifers, sensitive habitats and productive agricultural lands.

1.4 India and Bangladesh.

Low density production of *P. monodon* has been demonstrated in inland areas of India (Athithan et al., 2001). According to Nandeesha (2000), the production of *P. monodon* in freshwater arose through increased legislative pressure on coastal prawn farming and concerns over disease curtailing industry growth. Farmers in Bangladesh and India have recently been experimenting with production of *P. monodon* in freshwater either as a standalone crop or in conjunction with the giant freshwater prawn, *Macrobrachium rosenbergii.* According to figures supplied by the Marine Products Export Development Authority, some 16,000ha were utilised for *P. monodon* production in 2000-01 (Nandeesha, 2000). This had increased rapidly from just over 6000ha in 1997-98. Yields from such systems are typically less than 1t/ha.

Both low salinity groundwater or seawater transported from coastal areas is used to acclimate black tiger prawn postlarvae obtained from local hatcheries or wild seedstock suppliers. The acclimation rates vary from 8 to 30 days but once in the ponds, salinities will reach 0ppt by the end of the culture period, which may last up to 150 days. Reporting good survivals and growth, the prospects for freshwater production of marine prawns are positive, but the risk of relying on one species has prompted some farmers to alternate prawn production with fish culture.

In Bangladesh, industry assistance projects aimed at improving the livelihood and economic security of small-scale and marginal farmers has seen *P. monodon* culture in freshwater systems increase farm incomes and opportunity. Referred to as 'ghers', the salinity in these large extensive ponds reaches as low as 1.2ppt (Wahab, 2003). Again, due to their extensive nature, these systems do not produce large yields per hectare. Most are fed grains and other supplemental feeds, and are lightly stocked. Recent figures indicate that ghers returning less than 1t/ha have no deleterious effect on the environment and act as sinks for nutrients and solid wastes (Wahab, 2003).

1.5 Australian Inland Saline Aquaculture

In Australia, potential exists to develop a sustainable inland prawn farming sector using existing groundwater resources. The land and water resources required to develop such farms can vary greatly, but will be largely driven by water availability, quality and potential for environmental benefit. In other states, inland production of marine species using saline groundwater has largely focused on the use of brackish or saline groundwater with salinities close to that of seawater for the production of marine finfish and aquatic plants (Allen et al., 2001).

In Western Australia, the 'Outback Oceans' project was initiated to evaluate the feasibility of growing trout in farm dams over the winter months when water temperatures are favourable. Fish are stocked extensively early in the year as water temperatures cool and are harvested from September through to October as temperatures begin to rise above 20°C. In 2001-02, up to 200 farmers participated in this project that produced some 24t of trout. At present new production systems are being developed to make such activities more productive and reliable.

In South Australia, the use of saline groundwater for aquaculture ranges from the growout of barramundi (*Lates calcarifer*) to hatchery production of marine finfish such as yellowtail kingfish (*Seriola lalandi*), mulloway (*Argyrosomus hololepidotus*), whiting (*Sillaginodes punctata*) and others (Hutchison, 1999). Research into the production of marine finfish, plants and molluscs in interception schemes near Waikerie will continue with new facilities to be built in 2004.

In Victoria, saline groundwater is being used for the production of salt and brine shrimp (artemia). At present this is the only commercial operation in the state using saline groundwater specifically for aquaculture. However, the Victorian Department of Primary Industries is conducting research into novel technologies for the commercial production of various finfish and marine crustaceans using saline groundwater.

In New South Wales, irrigators have established large engineering schemes that intercept, hold and evaporate saline groundwater in order to lower the surrounding water table. This has created large saline storages which have potential to supply saline water for aquaculture. At present the potential for aquaculture is being assessed using facilities established at the Wakool Salinity Control Scheme in southern NSW. Here researchers are investigating the potential for marine and freshwater finfish production as well as prawn production in such schemes.

At the Northern Territory University, saline groundwater has been successfully used as the water supply for a trochus hatchery. Pilot-scale production trials of *Dunaliella salina*, a micro-algae high in carotenoids, have also been successfully conducted in Alice Springs but were never commercialised. Some trials have also been conducted on the suitability of saline groundwater for *P. monodon* production. The present status of these trials is unknown.

Environmental pressure on the coastal prawn industry, the ability of marine prawns to grow at a range of salinities, and clear global examples of successful inland prawn farming has prompted interest in the potential for development of an inland prawn industry in Queensland. While potential exists to develop these farms in water with salinities equal to that of seawater, the majority of groundwater in Queensland is much lower in salinity. As mentioned previously over two thirds of inland farms in the USA, China, Thailand and South America utilise water that is below 5ppt (Boyd and

Thunjai, 2003). It is likely that inland prawn farming in Queensland would develop using low salinity groundwater as a function of opportunity, availability and benefit.

1.6 Fresh, Brackish and Saline Groundwater

Salinity is a measure of soluble salts in or on soils, and in water (ANZECC and ARMCANZ, 2000). It is a general term used to describe the presence of mineral salts that predominate in the forms of sodium chloride, magnesium, calcium sulfates and bicarbonates. It is important to define what constitutes saline groundwater and what might be considered brackish or merely fresh groundwater as inland production of marine prawns does not rely on the presence of 'saline' waters.

Most prawn culture has traditionally occurred in coastal areas in full strength seawater to brackish waters with salinities above 10ppt. Seawater has a conductivity approaching 55,000µS/cm or around 35ppt. Boyd (2002) defines low-salinity prawn culture as that which occurs where the salinity does not normally rise above 10ppt and freshwater culture is that which occurs at 1ppt or less. Good quality drinking water has a total dissolved solids level of <500mg/L (approximately 800µS/cm or 0.5ppt).

As stated previously, inland areas of Australia are not necessarily 'freshwater' areas. This is not unusual as surface waters and soils in semi-arid to arid regions in many parts of the world are typically saline (Boyd, 2002). Saline aquifers in many regions overlay freshwater aquifers separated by an impervious stratum. As an example, the Winton and Mackunda sub artesian aquifer formations in western Queensland cover some 445,000 sq km and overlie the great artesian basin which generally lies too deep to access (Wiggins and Larsen, 2001). The water from this subartesian formation ranges in salinity from 550 to 25,000 µS/cm. Finding 'fresh' groundwater in this region is difficult with only 3% of bores delivering water suitable for human consumption. Most water is only useful for stock watering and general domestic use (Wiggins and Larsen, 2001).

In terrestrial agriculture, the salinity of water is generally rated according to plant tolerance groupings of very low, low, medium, high, very high and extreme tolerance (Table 1.1). These groupings are intended as a guide only because other factors such as soil characteristics, climate, plant species and irrigation management must be considered when determining the suitability of water for irrigation (ANZECC and ARMCANZ, 2000).

Table 1.1. Soil and water salinity criteria based on plant salt tolerance groupings[a].

Plant salt tolerance groupings	Water salinity rating	Average root zone salinity, EC (µS/cm)	Salinity as expressed in ppt[b]
Sensitive crops	Very low	<650	<0.41
Moderately sensitive crops	Low	650–1,300	0.4 – 0.82
Moderately tolerant crops	Medium	1,300–2,900	0.82 – 1.83
Tolerant crops	High	2,900–5,200	1.83 – 3.28
Very tolerant crops	Very high	5,200–6,100	3.28 –3.84
Generally too saline	Extreme	>6,100	>3.84

[a] Adapted from ANZECC and ARMCANZ (2000).
[b] Conversion of µS/m to ppt by multiplying salinity by 0.00063.

Boyd (2002) proposed that for the purposes of inland prawn farming 'freshwater' should be defined as that which has a salinity of 1.0ppt (<1,500 μS/cm) or less. Such water would be classed as typically low risk water for irrigation and can be used on moderately salt sensitive crops (Table 1.1). Boyd (2003) also proposed that 'low-salinity' prawn culture should refer to that which occurs in water of 10ppt (16,000 μS/cm) or less. However, any water above 3.8ppt would be classed as generally too saline for agriculture. Indeed once salinity passes 1.8ppt it could only be used on salt tolerant crops. Water that falls within the range of 0.4 to 1.8ppt would be classed as low to medium salinity and would be suitable for moderately sensitive crops. It is this water that has most potential for direct integration of black tiger prawn production and terrestrial farming in Queensland. Stand alone or 'zero-discharge' systems are likely when higher salinity waters are used.

1.7 Water Chemistry and Supply.

Groundwater may differ significantly in terms of its relative ionic composition compared to seawater (Boyd, 2001). Most saline groundwater is deficient in potassium although other key ions such as sodium, chloride, calcium and magnesium can also vary considerably depending on the aquifer. Significant variations in the composition of groundwater are often observed between bores that lie in close proximity to each other. This occurs because the aquifers from which they draw, while not necessarily distinct, often exist between different geological strata. The transfer of ions between the different clay particles within the strata and the water within the aquifer results in elements reaching an equilibrium that can be highly localised (Price, 1996). These waters also have potential to behave differently under culture conditions. Low salinity water can also react with bottom soils, significantly affecting the ionic composition of water held in open ponds (Boyd, 1995).

Major ion deficiencies in crustaceans can have serious physiological consequences ranging from stunted or poor growth through to asphyxiation, oedema and death. Potassium has an essential role in regulating sodium and therefore fluid balance within the hemolymph (McGraw et al., 2002). Potassium deficiency has been identified as a major factor for inland prawn farm operations which rely on groundwater (McGraw and Scarpa, 2002, Saoud et al., 2003). In the USA, better survival and yield has been obtained by inland farmers who actively supplement their water with mineral salts such as calcium sulphate (gypsum), potassium chloride (potash) and magnesium sulphate (Epsom salts) (Smith and Lawrence, 1990; Boyd and Teichert-Coddington, 2001; Boyd, 2002). .

Australian groundwater is typically deficient in potassium. Studies using marine finfish such as snapper and mulloway have identified that this can be readily addressed through the addition of muriate of potash (Fielder et al., 2001). No studies under Australian conditions are available on the short and long term effects of potassium supplementation on the survival and growth of marine crustaceans such as the black tiger prawn.

1.8 Acclimation and Growth

Growth of species such as *P. monodon* and *L. vannamei* have been shown to be unaffected by salinities as low as 2ppt (Samocha et al, 1998). Other species such as *P. merguiensis* have been found to be less tolerant of salinity change with significant mortality occurring below 7ppt (Zacharia and Kakati, 2002). The stage of gill development (as a function of age and therefore osmoregulatory capacity) will also determine the ability of postlarvae (PL) to adapt to low salinity environments (Saoud et al., 2003). Full gill development in *L. vannamei* PL is usually achieved by day 12 (McGraw et al., 2002) and in *P. monodon* by day 15 (Cawthorne et al., 1983).

Younger PL do not cope as well with low salinity and suffer significant levels of mortality (Rosas, et. al., 1999; Saoud et al., 2003).

As well as not being tolerant to large fluctuations in salinity when very young, Penaeid prawns lose their ability to cope with large changes in salinity the older they get (Saoud et al., 2003). Previous studies have demonstrated that *P. monodon* juveniles do not cope as well with salinity change compared to PL (Pantastico and Oliveros, 1980; Cawthorne et al., 1983). This age dependent ability to cope with salinity change determines the optimal age at which a species of prawn can be adapted from seawater to low salinity groundwater.

Survival during stocking of PL is also dependent on the rate and method of acclimation. Pantastico and Oliveros (1980) demonstrated that the rate of acclimation of *P. monodon* PL had a significant influence on survival. Catedral et al, (1977), observed that *P. monodon* PL can tolerate salinity changes of 10 – 20ppt without acclimation, but to achieve high survival at salinities as low as 3ppt, slower rates of acclimation are required. McGraw et al. (2002), found that for *L. vannamei* PL, the rate of salinity reduction was not as important as the age of the animal at the time of acclimation.

The particular strain of a species has also been proposed to influence acclimation success and growth in low salinity waters. In the case of *L. vannamei*, Ecuadorian stock used in low salinity growth trials has performed better than Mexican stock used in other trials (Huang, 1983; Bray, 1994; Samocha et al., 1998).

Other environmental factors such as temperature and water quality can also play a significant role in acclimation of a particular species (Harpaz and Karplus, 1991). Tsuzuki et al. (2000) demonstrated that temperature, ontogenetic development and salinity change can act synergistically to affect survival in the Brazilian pink shrimp (*Farfantepenaeus paulensis*). Water quality can also affect survival at low salinities as juvenile *P. monodon* are more sensitive to ammonia, nitrite and nitrate toxicity at salinities lower than normal seawater (Lin and Chin, 2003; Tsai and Chen 2002). Acclimation strategies must therefore provide relatively better water quality at lower salinities than would otherwise be required in higher saline environments.

The acclimation of postlarvae (PL) to low salinity inland waters can be used as an indicator of the suitability of that water for growout (Saoud et al., 2003). Those PL that acclimate well to low salinity waters have also been shown to grow well under laboratory conditions (Cawthorne et al., 1980; Harpaz and Karplus 1991; Saoud et al., 2003). The acclimation, survival and growth of the Australian black tiger prawn must also be established in a range of groundwater types from various sources across the state in order to determine what prospects exist for the production of this species in inland regions of Queensland.

1.9 Biosecurity

An additional benefit of inland groundwater culture of marine crustaceans is the innate biosecurity advantage of such culture systems. Groundwater free of marine pathogens has been used by American producers to develop certified 'organic', 'chemical free' or 'antibiotic free' farms. It use has enabled growers to source and maintain specific pathogen free (SPF) stock and in some cases initiate the establishment of inland biosecure or 'high health' hatcheries.

The most significant disease threat in most prawn producing countries is White Spot Syndrome Virus (WSSV). This virus has caused significant losses for prawn producers in many Asian and South American countries. The virus can be observed in its latter stages to form white spots on the prawn's carapace. Mortality usually follows within three to 10 days after onset of external symptoms. Other diseases such as yellow head virus, taura syndrome and Gill Associated Virus (GAV) are further examples of diseases which account for significant losses for the prawn industry world wide. Of all these diseases only GAV is known to exist in Australia.

Gill Associated Virus is endemic to *P. monodon* broodstock captured from Australia's north-east coast. In healthy animals GAV is present at low levels and is seemingly innocuous. However, when the prawn is stressed, such as through poor water quality, being held at high density or infection by another disease, GAV multiplies within the animal's cephalothorax and the infection becomes lethal. If a source of GAV free broodstock were identified in Australia, inland farms may provide the opportunity to develop 'high health' hatcheries for the supply of SPF prawn PL. Those farms stocked with GAV free stock would also have the potential to be used as a source of SPF broodstock if domestication technologies were developed.

1.10 Environmental Management

The use of saline, brackish and even low salinity groundwater for inland aquaculture poses important issues concerning deleterious impacts of such activities on the environment. However, it is well recognised that salinity is a major environmental challenge facing Australia. The development of aquaculture could be considered as a productive use of salt affected groundwater and soils. However, industry development must be managed in a way that will not contribute to existing salinity problems in these areas.

Boyd (2001) observed that the benefits of inland prawn culture are 'multifold' and include the opportunity to diversify land use options, limit impacts of prawn farming on the environment, reduce incidence of disease, simplify farm logistics and improve control over water supply and use. However, the same author views the major constraint to inland prawn farming as being related entirely to the possibility of salinisation of soils, underlying freshwater aquifers and local streams. The author states that inland prawn farming can be conducted without causing adverse environmental effects provided that good farm development and management practices are observed. Such practices would require that ponds do not seep into freshwater aquifers, streams or non-saline soils; pond culture water is reused and not discharged off farm; pond sediment is dried and either used to re-shape ponds or deposited in contained spoil pits; vegetative barriers and piezometer tubes are installed around the farm's perimeter to monitor and manage salt intrusion; and soils in the bottom of abandoned ponds and surrounding areas be reclaimed through treatment with gypsum.

These conditions can be readily met through appropriate site selection, pond design, construction, and management. Ponds can be lined with inert plastic liners to prevent seepage although for low and semi-intensive production, it is usually more economic to build earthen ponds. Earthen ponds should be constructed with soil whose clay content is over 70%. Adequately compacted pond dykes and bottoms should seep no more than 0.1mm per day.

Pond operations that contain or treat and re-use their effluent are referred to as 'zero-discharge' or 'recirculated' farms. As discussed previously, operations such as OceanBoy Farms in Florida use fully lined ponds and completely recirculate their waters. However, not all inland prawn farms contain and treat their effluent waters. In some cases, inland prawn farms are integrated with irrigated agriculture (Figures 1.5, 1.6). McIntosh and Fitzsimmons (2003) demonstrated that effluent from an inland prawn farm in Arizona with a salinity of 2.2ppt, could be used supply between 20 and 31% of the nitrogen fertilizer necessary for the production of salt tolerant crops such as wheat. These authors noted that while Arizona has a long history of

Figure 1.4 Desert Sweet Shrimp at Gila Bend in Arizona produces the white shrimp (*Litopenaeus vannamei*) using low salinity groundwater.

Figure 1.5 Effluent from low salinity inland prawn farms can be used for irrigation of salt tolerant crops such as sorghum, cotton, wheat and olives (McIntosh and Fitzsimmons, 2003). In this case effluent from prawn farming activities is being used to irrigate an olive crop.

groundwater use in the cotton, sorghum and grain industries, the high sodium levels in this groundwater would not necessarily make this a favourable activity in other regions. However, when the clay content of soil is low, as in the Arizona study (<7.7%), rain will wash this salt from the root zone and reduce the risk of damaging salt sensitive crops.

Potential exists to establish similar zero-discharge or integrated farms in Australia using clay or plastic lined ponds. These facilities might even be developed in association with salt interception and disposal schemes to make productive use of an otherwise problematic resource.

1.11 Inland Farming of Marine Prawns In Australia

Although recognised as the most favourable prospect for inland saline aquaculture in Australia (Allen and Fielder, 1999), research into commercial farming of marine prawns using inland saline waters is not well advanced. Ingram et al., (2002) reported that attempts to acclimate black tiger prawns to inland saline waters in Victoria failed. Reasons for this failure were likely related to the mineral imbalances that exist in inland saline waters.

Considering that Australia has more saline groundwater (relative to the amount of open land) than any other country, the potential for marine prawn farming in inland regions appears significant (McNeil, 2003). There are however several biophysical, economic and environmental constraints to such development that must be addressed. Primarily, the availability and suitability of water in regions favourable for open pond prawn production must be determined. This requires not only determination of the size of resource, but also involves testing the effectiveness of mineral supplementations, water quality management techniques, and effluent treatment systems on the growth and survival of black tiger prawns. Secondly, the economic case for the development of inland prawn farms must also be balanced against its technical and operational challenges.

1.12 Project Objectives

The objectives of this study were to address the primary technical issues associated with the use of groundwater for inland prawn production. Specifically the objectives of this project included:

- A review of data concerning groundwater use, suitability and availability in key regions in Queensland.
- Establishment of methods to permit the rapid acclimation and transfer of marine prawns to fresh and low salinity groundwater for growout.
- Ascertaining the mineral supplementation required to enable individual groundwater sources to be used for prawn culture.
- Comparing the growth of black tiger prawns in marine and inland saline waters.
- Conducting inland pond trials to investigate the technical issues concerning semi-intensive production of the black tiger prawn using groundwater.

The results of these studies and their implication for potential developers, managers of the environment, industry and the broader community are discussed in the following chapters.

2. Mapping Regional Groundwater Salinities

2.1 Objective

The purpose of this activity was to identify and collate known information concerning groundwater salinity in regional areas. The Department of Natural Resources and Mines (DNRM) groundwater database was used to access information concerning the salinity of individual bores in a number of key regions across Queensland. This activity was undertaken to focus future research and development effort within regions found to have high potential for inland saline aquaculture.

2.2 Methods

Several regions were identified as having potential for inland prawn farming because of the existence of bore infrastructure, a favourable climate, access to suitable land and recognised interest in farm diversification. Information concerning the salinity of individual bores from several regions was collated, ranked according to their salinity profile, expressed as electrical conductivity (EC), then grouped and plotted. These 'salinity maps' of bores were then used to highlight areas of significant potential within each region.

2.3 Results and Discussion

This study looked at several regions including the Burdekin, Rockhampton, Maryborough, Longreach, Emerald, Charleville, St George, Toowoomba and the Lockyer Valley. All of the regions have access to groundwater with salinities suitable for prawn farming. These regions are identified in Figure 2.1 and are shown in detail in Figures 2.2 to 2.10.

The Burdekin region near Ayr (Figure 2.2) is characterised by a large number of bores that were drilled primarily to irrigate the region's sugarcane crops. A large cluster of bores with conductivities in excess of 3,000µS/cm is located on the eastern bank of the Burdekin River near the township of Clare. In irrigation terms, the salinity of this water is generally considered as high and suitable for salt tolerant crops only (Table 1.1). Over 32% of bores in the study area recorded conductivities equal to, or in excess of, 3,000µS/cm. A further 16% of bores recorded conductivities between 1,500 and 3,000µS/cm. In clay soils it is recommended that the conductivity of water used to irrigate sugarcane should not exceed 1,400µS/cm (QNRM, 2003). Closer to the coast, saltwater intrusion from marine waters may explain the frequency of extremely high salinity bores in these areas.

In Rockhampton, similar clusters of bores with conductivities in excess of 3,000µS/cm are evident in association with most heavily irrigated areas (Figure 2.3). The two largest clusters of high salinity bores are located at Mt Larcom near Gladstone, and Wowan, south west of Mt Morgan. However, bores with conductivities in excess of 3,000µS/cm are also located west from Rockhampton to the townships of Richlands and Gogango. On a regional basis, conductivities in excess of 3,000µS/cm represent over 36% of all bores. An almost equal number of bores (33%) have conductivities greater than 1,500µS/cm but less than 3,000µS/cm. Of the remaining bores, almost 26% range between 500 and 1,500µS/cm while less than 5% have conductivities under 500µS/cm.

Little data was available for bores in the Maryborough and Childers regions (Figure 2.4). Although a large sugarcane area, most irrigation water is drawn from the Mary River and its tributaries. However, from the data available it is evident that groundwater in the Maryborough to Childers region is often highly saline with 32% of all bores having conductivities in excess of 3,000µS/cm.

In the central western region two types of bores are common. These are artesian and sub-artesian. The data presented in this study does not identify the bore type. Subartesian waters are the most likely source of water for prawn farming in this region as artesian waters are generally lacking in key elements and other parameters such as pH, hardness and alkalinity are often unsatisfactory. Of the bores mapped in this study less than 10% had conductivities in excess of 3,000µS/cm (Figure 2.5). Studies into the subartesian groundwater formations of the central west clearly identify a water source that is large in volume but has limited recharge capacity and is deteriorating in quality. This deterioration in quality is characterised by increasing salinity. Of the 2,700 thousand bores that have been drilled into the Winton-McKundle sub-arteasian formation, almost 65% have been abandoned because of rising salinity and decreasing yields (Wiggins and Larsen, 2001). Since the 1970's there has been a 3 fold increase in the conductivity of bores in this area resulting in a decrease in the number bores suitable for human consumption from 54% to just 3% in 2000.

The Emerald region is characterised by intensive irrigation and its major crops are cotton and cereal grains. Most irrigation water used in this region is supplied by Fairburn Dam. However, a number of bores are used to supplement this supply. Of the 418 bore records available for the Emerald region, 37% were found to have conductivities above 3,000µS/cm (Figure 2.6). Most of these bores are readily distinguished as separate clusters of bores around Emerald itself as well as the townships of Rubyvale and Sapphire. Of the remaining bores 57% had conductivities between 500 and 3,000µS/cm.

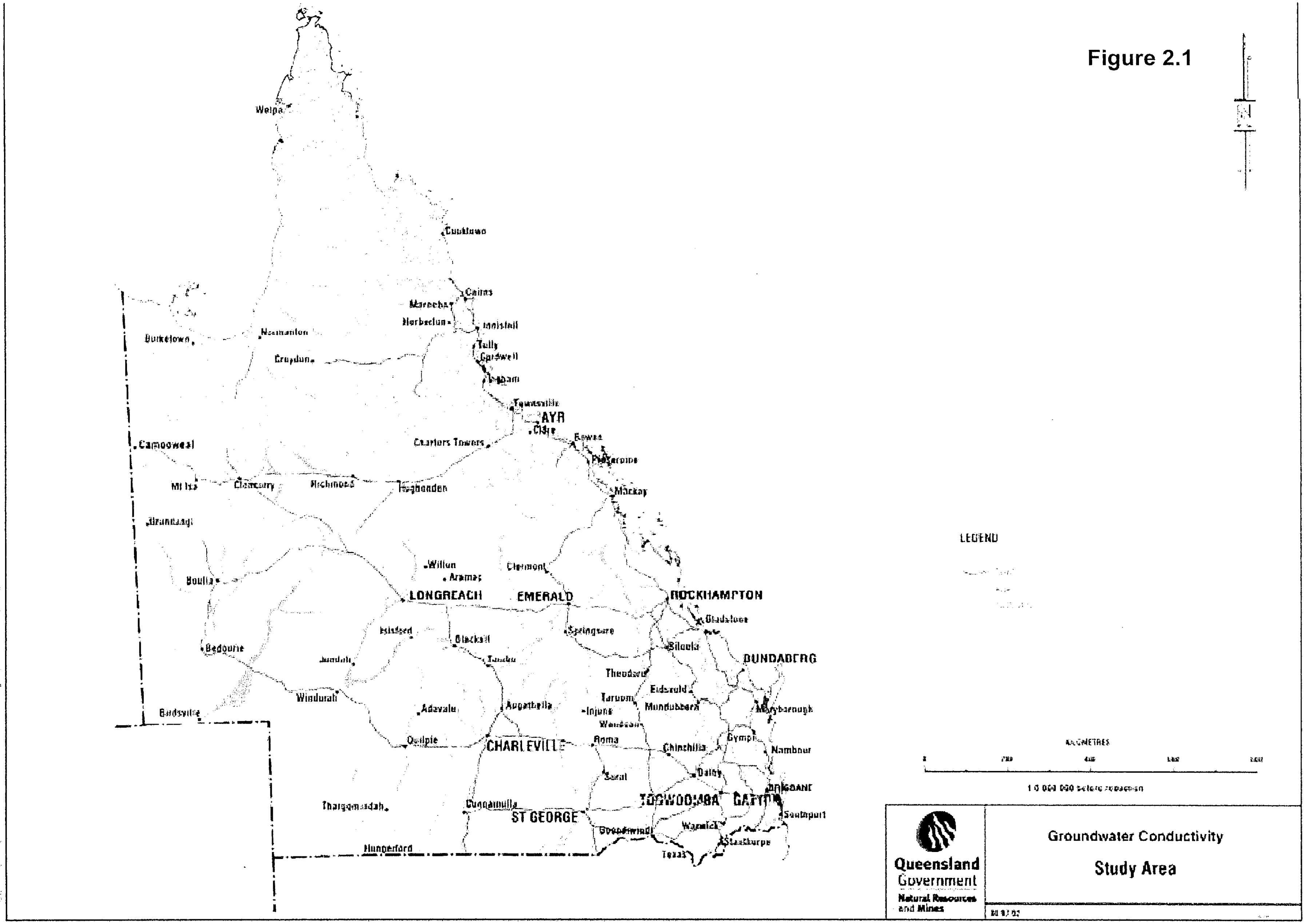
As with the Longreach and Emerald regions, the number of bores is small in the Charleville (Figure 2.7) and St George regions (Figure 2.8) compared to areas with greater reliance on and regulation of groundwater. In Charleville 90% of all bore water recorded maximal conductivities of between 500 and 1,500µS/cm. Only 5% of bores had conductivities in excess of 1,500µS/cm. In contrast, the frequency of salt affected bores in St George was proportionally high. Of the 104 bores identified in St George, 42% had EC's in excess of 3,000µS/cm.

On the Darling Downs irrigated cotton and grains production relies heavily on the availability of groundwater. In this study, the records of almost 1,500 bores covering an area from Chinchilla to Bowenville, indicate that up to 44% have EC's in excess of 3,000µS/cm (Figure 2.9). A further 22% of bores have EC's of between 1,700 and 3,000µS/cm, while just 10% of bores might be considered to satisfy potable freshwater standards of 800µS/cm or less.

Similarly, salinities of irrigation bores in the Lockyer Valley region were also high (Figure 2.10). Of the almost 2,200 bores considered in this study, 92% had conductivities over 500µS/cm. Of these, 32% had conductivities between 1,500 and 3,000µS/cm while 27% exceeded 3,000µS/cm.

Of the almost 8,500 individual bores considered in this study, 33% had conductivities in excess of 3,000µS/cm. A further 27% had conductivities above 1,500µS/cm but less than 3,000µS/cm. The black tiger prawn (*P. monodon*) has been grown successfully in water considered fresh enough to meet potable freshwater standards (Pantastico and Oliveros, 1980; Athithan et al., 2001). Bores with salinities greater than 800µS/cm represent over 81% of those considered here. This data indicates that the conductivity of groundwater from existing bores in many regions is sufficiently salty for inland production of black tiger prawns. Additional factors such as yields from each bore and water chemistry will also have to be assessed before deeming a bore suitable for this type of aquaculture.

Figure 2.1



Queensland Government
Natural Resources and Mines

Groundwater Conductivity

Study Area

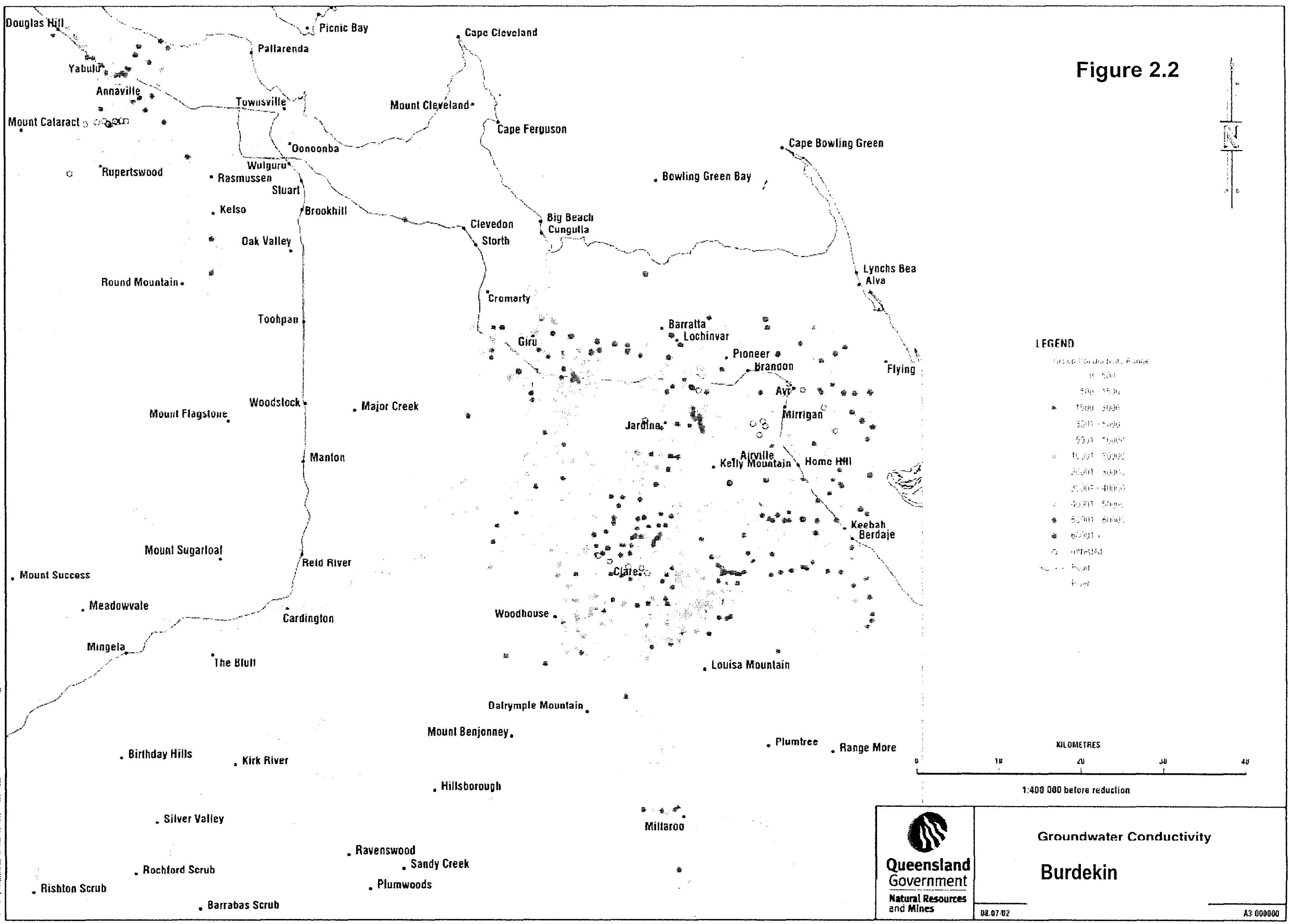


Figure 2.2
N
Douglas Hill
Picnic Bay
Cape Cleveland
Pallarenda
Yabulu
Annaville
Townsville
Mount Cleveland
Mount Cataract
Cape Ferguson
Cape Bowling Green
Oonoonba
Rupertswood
Wulguru
Rasmussen
Stuart
Bowling Green Bay
Kelso
Brookhill
Clevedon
Big Beach
Cungulla
Oak Valley
Storth
Lynchs Bea
Alva
Round Mountain
Cromarty
Toohpan
Barratta
Lochinvar
LEGEND
Giru
Pioneer
Brandon
Flying
Woodstock
Major Creek
Mount Flagstone
Ayr
Mirrigan
Jardina
Airville
Kelly Mountain
Home Hill
Manton
Keebah
Berdaje
Mount Sugarloaf
Reid River
Mount Success
Clare
Meadowvale
Cardington
Woodhouse
Mingela
The Bluff
Louisa Mountain
Dalrymple Mountain
Mount Benjonney
Plumtree
Range More
KILOMETRES
0
10
20
30
40
1:400 000 before reduction
Birthday Hills
Kirk River
Hillsborough
Silver Valley
Millaroo
Groundwater Conductivity
Burdekin
Ravenswood
Sandy Creek
Rochford Scrub
Plumwoods
Rishton Scrub
Barrabas Scrub
Queensland Government
Natural Resources and Mines
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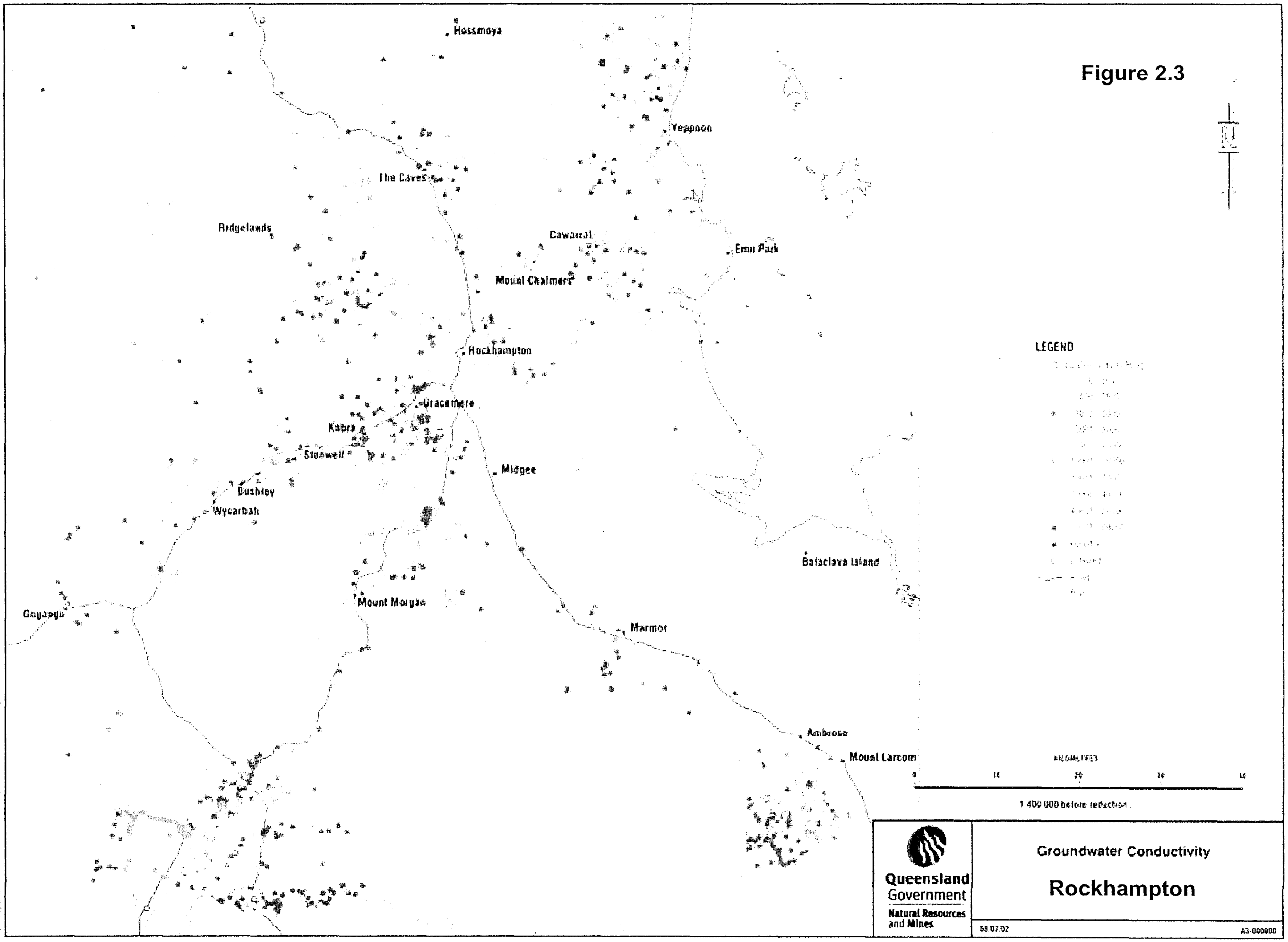
Figure 2.3
Rossmoya
Yeppoon
The Caves
Ridgelands
Cawarral
Emu Park
Mount Chalmers
Rockhampton
Gracemere
Kabra
Stanwell
Midgee
Bushley
Wycarbah
Balaclava Island
Mount Morgan
Gogango
Marmor
Ambrose
Mount Larcom
LEGEND
1 400 000 before reduction
Groundwater Conductivity
Rockhampton
Queensland Government
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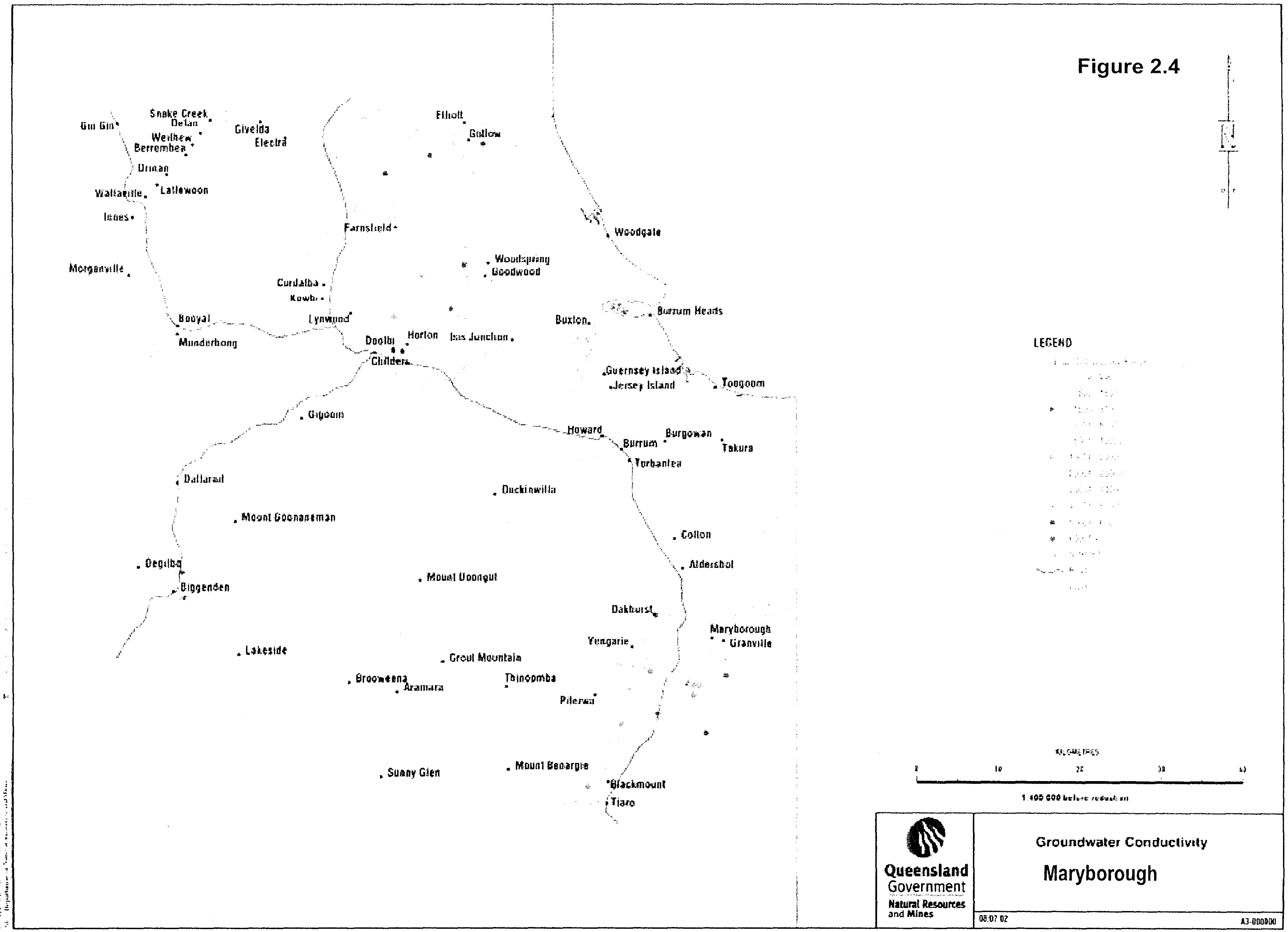
Figure 2.4
Gin Gin
Snake Creek
Delan
Givelda
Electra
Woolhew
Berrembea
Elliott
Gillow
Walla
Lallewoon
Wallaville
Innes
Morganville
Farnsfield
Woodgate
Woodspring
Goodwood
Cordalba
Booyal
Lynwood
Mungerbong
Doolbi
Horton
Isis Junction
Childers
Buxton
Burrum Heads
Guernsey Island
Jersey Island
Toogoom
Gigoom
Howard
Burrum
Burgowan
Takura
Torbanlea
Dallarnil
Duckinwilla
Mount Goonaneman
Colton
Aldershot
Degilbo
Biggenden
Mount Doongul
Dakhurst
Maryborough
Granville
Yengarie
Lakeside
Grout Mountain
Brooweena
Aramara
Thinoomba
Pilerwa
Sunny Glen
Mount Benarige
Blackmount
Tiaro
LEGEND
Groundwater Conductivity
Maryborough
Queensland Government
Natural Resources and Mines
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Figure 2.5

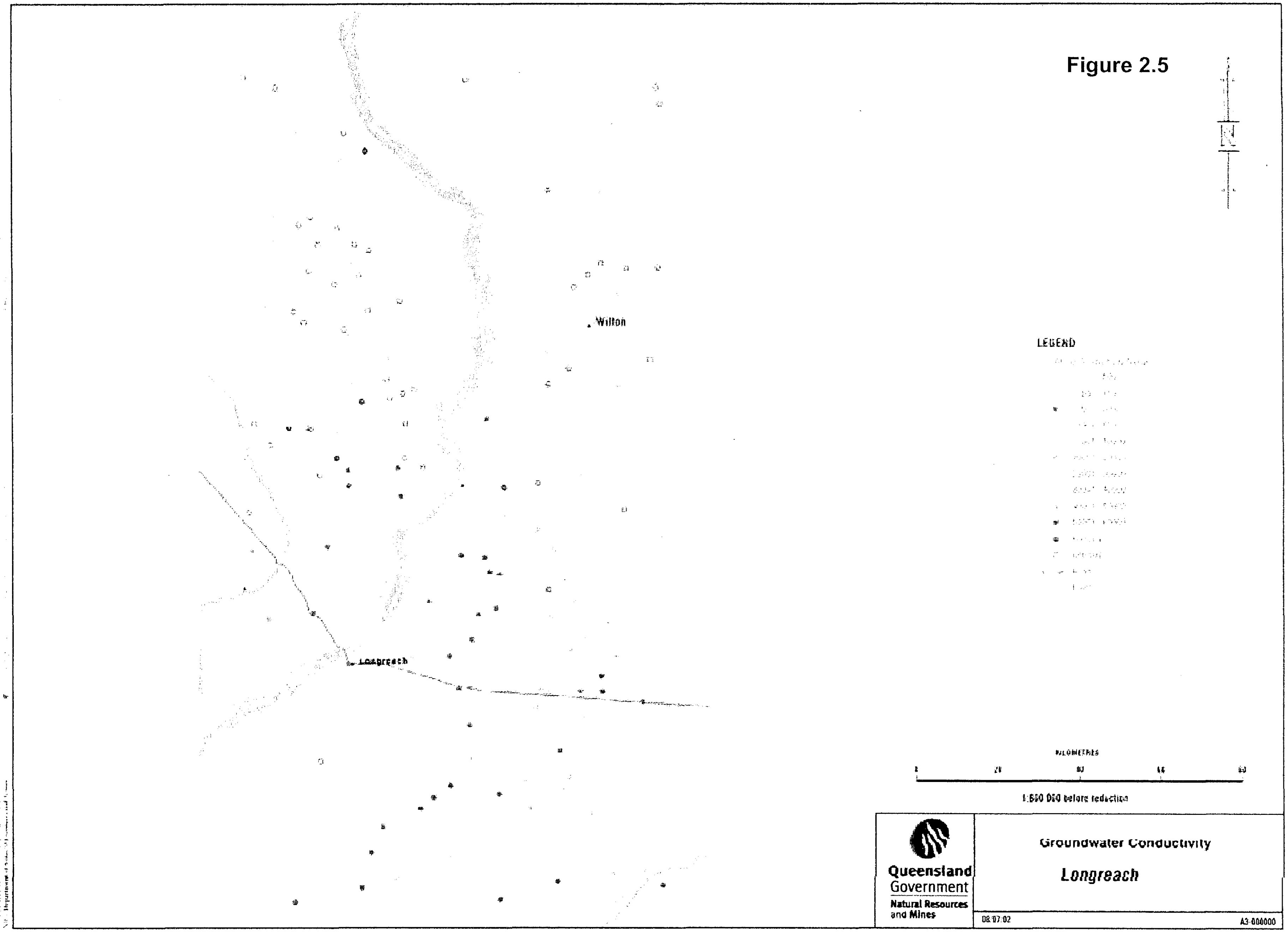
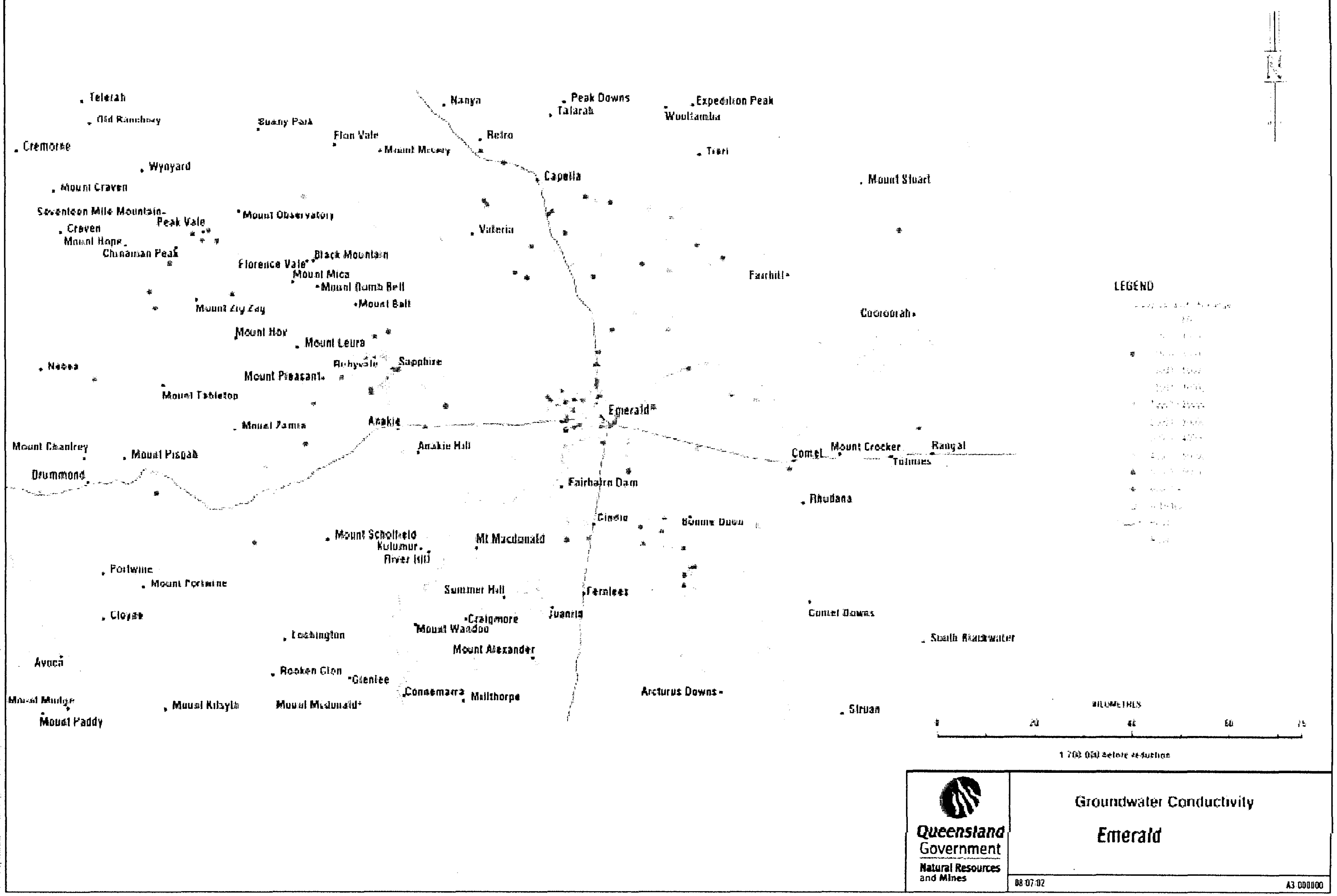

Wilton
Longreach
LEGEND
KILOMETRES
1:650 000 before reduction
Queensland Government
Natural Resources and Mines
Groundwater Conductivity
Longreach
08.07.02
A3-000000

Figure 2.6


Telerah
Cremorne
Wynyard
Mount Craven
Seventeen Mile Mountain
Peak Vale
Craven
Chinaman Peak
Mount Observatory
Florence Vale
Black Mountain
Mount Mica
Mount Dumb Bell
Mount Ball
Mount Zig Zag
Mount Hay
Mount Leura
Sapphire
Mount Pleasant
Mount Tableton
Anakie
Anakie Hill
Mount Pisgah
Drummond
Nanya
Retro
Peak Downs
Talarah
Expedition Peak
Capella
Mount Stuart
Vaterie
Fairhill
Emerald
Fairbairn Dam
Comet
Mount Crocker
Rangal
Rhodana
Mount Schollield
Mt Macdonald
Portwine
Mount Portwine
Summer Hill
Fernlees
Cloyne
Craigmore
Mount Wandoo
Mount Alexander
Lochington
Avoca
Comet Downs
South Blackwater
Glenlee
Connemarra
Millthorpe
Arcturus Downs
Mount Kilsyth
Mount Paddy
Stuart
LEGEND
KILOMETRES
Queensland Government
Natural Resources and Mines
Groundwater Conductivity
Emerald

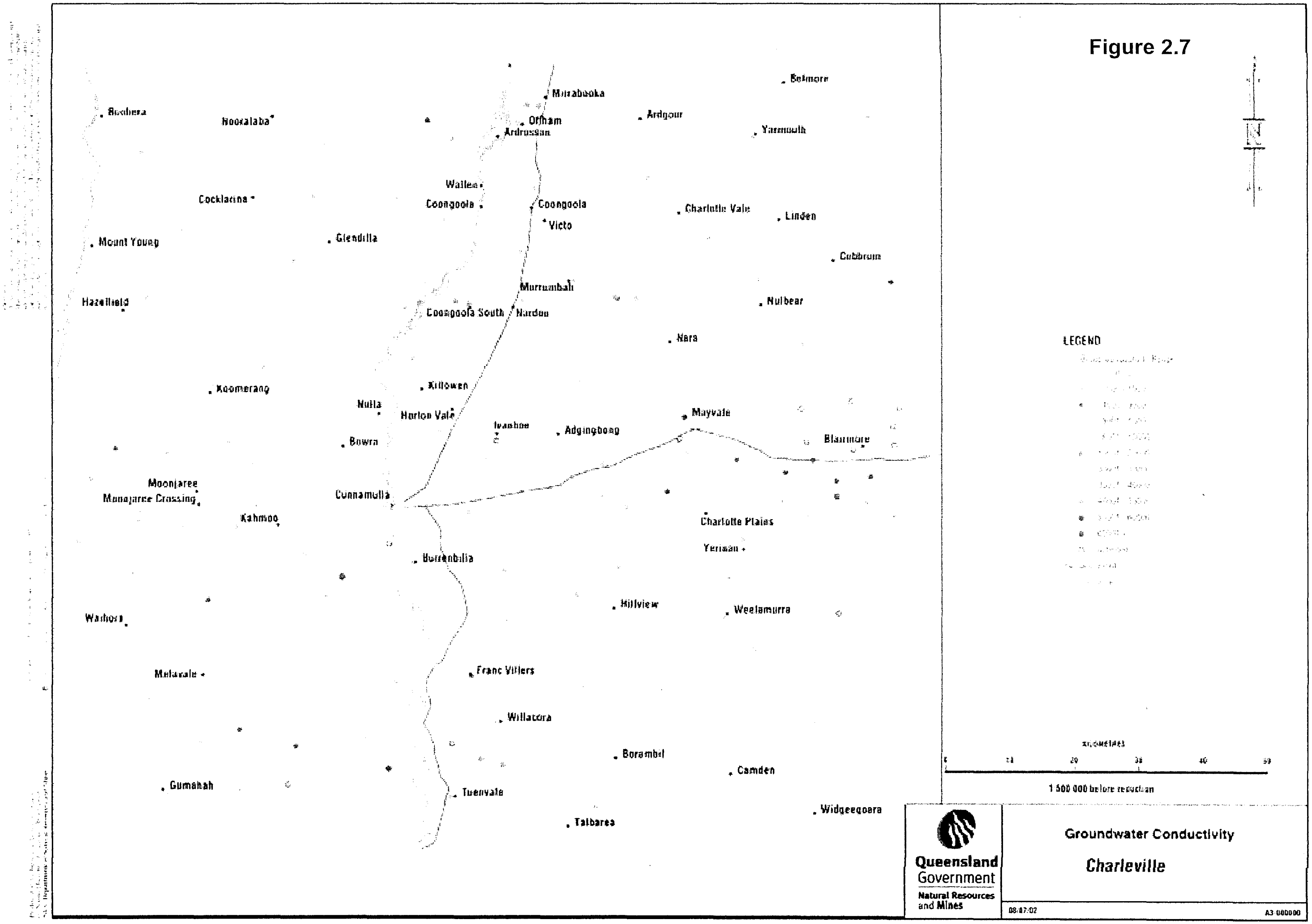


Figure 2.7
LEGEND
Groundwater Conductivity
Charleville
Queensland Government
Natural Resources and Mines
Cunnamulla
Mayvale
Charlotte Plains
Burrenbilla
Franc Villers
Willacora
Borambil
Camden
Tuenvale
Talbarea
Widgeegoara
Gumahah
Wahora
Kahmoo
Koomerang
Glendilla
Cocklarina
Nooralaba
Mount Young
Hazelfield
Nulbear
Linden
Ardgour
Belmore
Hillview
Weelamurra
Adgingbong
Bowra
Nulla
Killowen
Murrumbah
Nardoo
Coongoola
Victo
Charlotte Vale
Cubbrum
Blairmore
Moonjaree
Moonjaree Crossing
Orfham
Ardrussan
Mirabooka
Wallen
Yarmouth
Nara
Horton Vale
Ivanhoe
Coongoola South
Yerisan

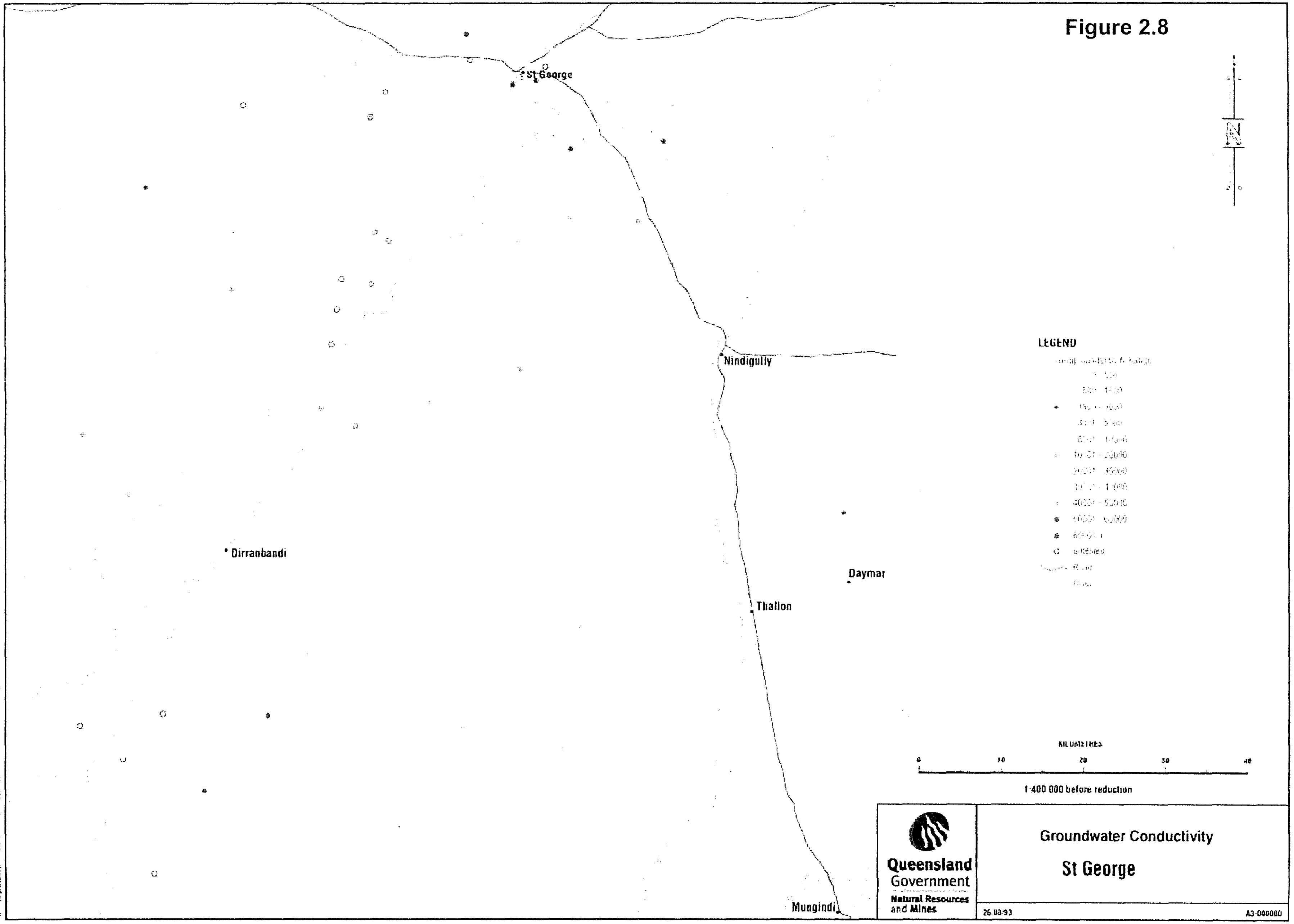
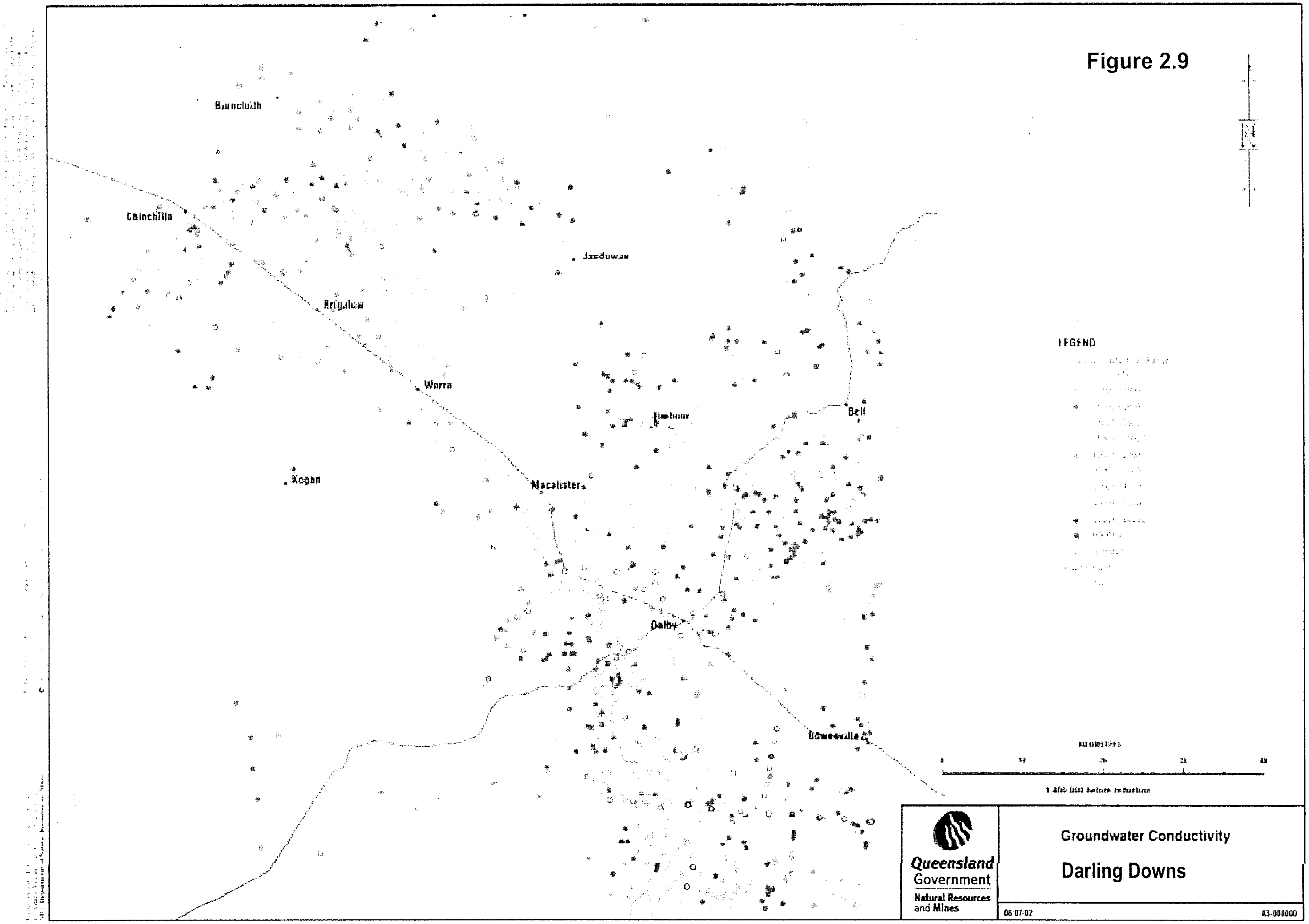


Figure 2.8
St George
Nindigully
Dirranbandi
Daymar
Thallon
Mungindi
LEGEND
KILOMETRES
0
10
20
30
40
1:400 000 before reduction
Queensland
Government
Natural Resources
and Mines
Groundwater Conductivity
St George
26.08.93
A3-000000



Inland Prawn Farming - Studies into the Potential for Inland Marine Prawn Farming in Queensland

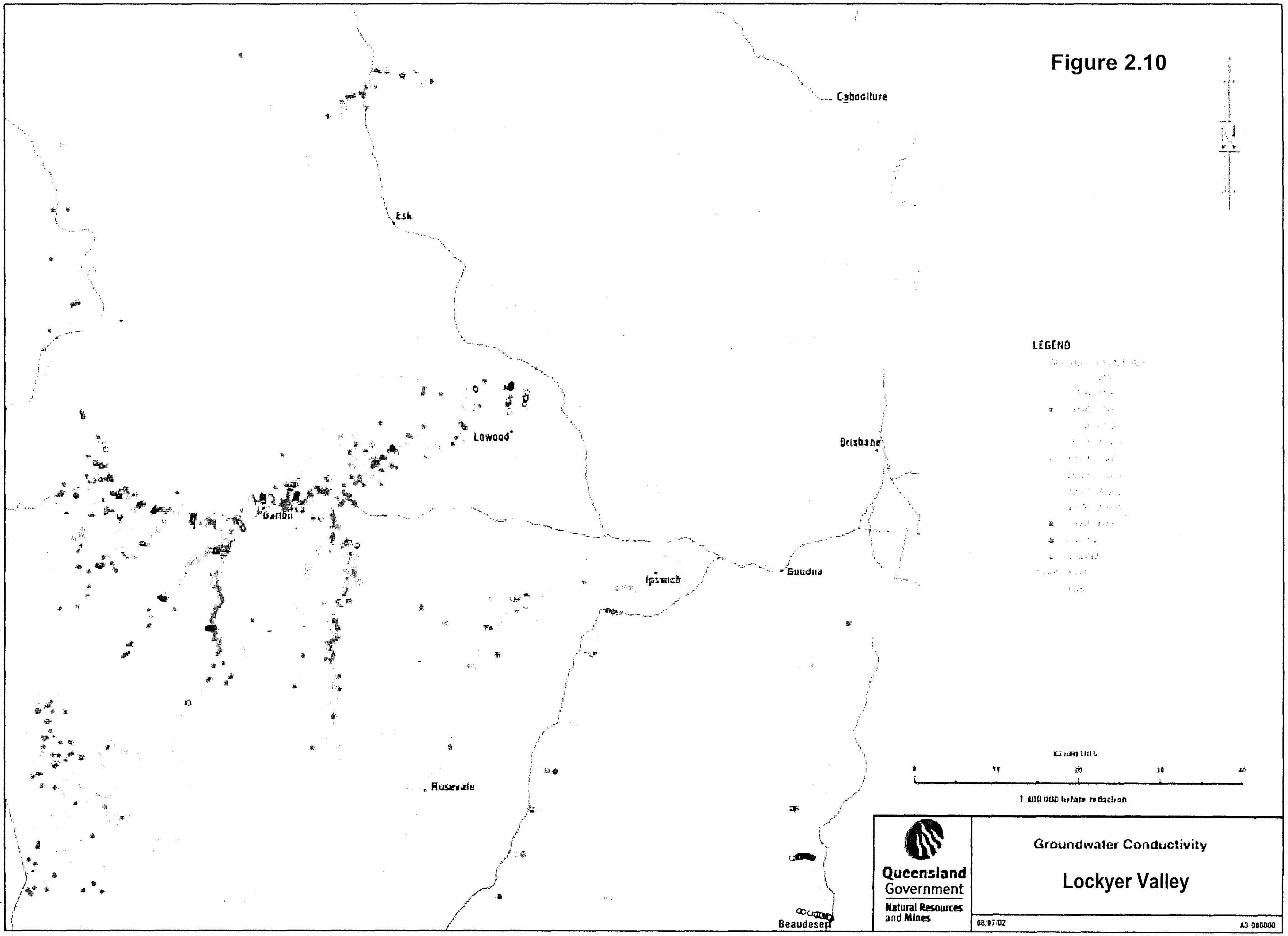


Figure 2.10
Caboolture
Esk
LEGEND
Lowood
Brisbane
Gatton
Ipswich
Goodna
Rosewale
Beaudesert
1 400 000 before reduction
Queensland
Government
Natural Resources
and Mines
Groundwater Conductivity
Lockyer Valley
08/07/02

3. Water Analysis

3.1 Objectives

These trials were undertaken in order to identify the characteristics of individual water samples from a number of regions in Queensland. As these waters are often deficient in key elements, have excessive concentrations of some compounds, and can vary significantly in quality, this study sought to determine what water chemistry manipulation was required to make them suitable for prawn survival.

3.2 Methods

The first step involved the collecting and analysing water from 23 study sites. These sties are identified in Table 3.1.

Table 3.1. Region, locality and sample identifier for water samples analysed.

Region	Locality	Sample Identifier
South-East	Lockyer Valley	LV1, LV2, LV3, LV4
	Tiaro	SE1, SE2, SE3
Southern	Darling Downs	DD1, DD2, DD3
South-West	Surat Basin	SB1
	Mitchell	SW1
Central-West	Longreach	CW1, CW2, CW3, CW4
North-West	Mt Isa	NW1
North Coastal	Burdekin	B1, B2, B3, B4, B5, B6

Individual bore profiles can change over time and so all samples were tested in order to determine the present salinity level and concentration of major ions (Table 3.2). Samples were collected in 500ml bottles and sent directly for analysis either at the Department of Natural Resources and Mines, Natural Resource Chemistry Centre, (Indooroopilly, Brisbane) or at the Australian Laboratory Services P/L (Stafford, Brisbane).

To facilitate the comparison of groundwater ion balances and those of seawater, the concentration of major cations (sodium, magnesium, calcium, potassium), anions (chloride, sulphate, carbonate, bicarbonate, hydroxide, fluoride, nitrate) and others (eg. phosphorous and iron) were compared to that of seawater diluted to the equivalent conductivity (Table 3.3). The diluted seawater (DSW) value is the expected concentration of an individual property or ion at the same salinity as the groundwater sample. The relative seawater balance (RSB) is a comparative measure of the actual concentration of a compound in the groundwater sample expressed as a percent of the DSW value:

RSB = [concentration in sample (mg/L) / concentration in DSW (mg/L)] * 100

Table 3.2 Composition of groundwater samples analysed in this study and used in subsequent bioassays, laboratory growth trials and pond based growth trials. Electrical conductivity presented in µs/cm while hardness, alkalinity (as CaCO3) and other ions presented as mg/L. Sample SWC refers to a seawater control.

Sample	pH	Conductivity	TDI	Total Hardness	Total Alkalinity	Residual alkalinity	Ca	Mg	Na	K	OH	CO_3	HCO_3	SO_4	Cl	F	NO_3	PO_4	Fe
SWC	8.20	52400	34920	6480	318	<0.01	409	1320	10500	398	0.00	12.9	362	2,780	19,080	<0.01	<0.01	<0.1	0.2
LV1	7.50	46700	27805	3666	167	<0.1	283	710	9285	63	<0.1	<0.1	204	1,450	15,810	<0.1	<0.1	-	-
LV2	7.50	14430	8468	1387	905	<0.1	113	265	2540	0	<0.1	<0.1	1,104	295	4,150	1.0	<0.1	-	-
LV3	8.40	2690	1380	1070	210	<0.1	97	200	170	4	<0.1	6.0	260	9	640	<0.1	0.9	<1	<0.1
LV4	7.80	13300	7530	1278	156	<0.1	141	222	2311	12	<0.1	<0.1	190	361	4,293	<0.1	<0.1	<1	
SE1	7.30	5710	3000	560	41	<0.1	62	98	1000	6	<0.1	<0.1	50	19	1,770	<0.1	<0.1	<1	<0.1
SE2	8.00	3580	1880	880	290	<0.1	140	130	480	1	<0.1	<0.1	360	71	700	<0.1	4.4	<1	<0.1
SE3	7.10	15570	8893	1691	735	<0.1	238	263	2650	0	<0.1	<0.1	897	275	4,570	<0.1	<0.1		
DD1	8.40	3870	2070	370	145	<0.1	46	61	690	6	<0.1	3.0	180	99	1,000	<0.1	<0.1	<1	<0.1
DD2	7.95	14100	8883	3330	553	<0.1	532	486	1960	23	<1	<1	553	667	4,540	0.1	0.1	<0.01	<0.01
DD3	8.30	10650	7069	1762	1610	<0.1	201	300	1710	22	<1	<1	1,964	622	2,250	-	-	-	-
SB1	8.15	7630	6208	80	1730	33.60	13	4	2060	24	<1	<1	1,730	1	1,990	6.3	<0.01	0.0	0.1
SW1	8.00	2170	1000	110	26	<0.1	41	1	400	3	<0.1	<0.1	32	110	430	<0.1	<0.1	<1	<0.1
CW1	8.60	3350	1750	62	105	0.80	12	8	660	5	<0.1	5.0	130	17	920	<0.1	<0.1	<1	<0.1
CW2	8.03	1720	1455	45	696	13.00	15	2	394	20	<1	<1	696	6	164	4.7	<0.01	0.0	0.3
CW3	8.40	14000	8130	540	64	<0.1	170	29	3020	10	<0.1	3.0	78	10	4,820	<0.1	<0.1	<1	<0.1
CW4	8.50	3740	1920	280	66	<0.1	78	20	670	11	<0.1	3.0	80	9	1,050	<0.1	5.7	<1	<0.1
NW1	7.53	3920	2535	1080	376	<0.1	193	145	443	11	<1	<1	376	337	946	0.6	1.1	0.0	<0.01
B1	8.10	4540	2794	1233	484	<0.1	166	198	503	1	<0.1	7.9	574	112	1,220	-	3.3	-	0.5
B2	6.80	20000	11754	4494	130	<0.1	676	682	2681	29	<0.1	0.1	158	1,064	6,464	-	0.0	-	0.0
B3	7.80	9700	6555	855	1022	3.10	83	158	2016	2	<0.1	8.6	1,229	182	2,867	-	0.0	-	0.0
B4	7.80	10500	6969	1214	142	<0.1	140	210	2150	61	<0.1	1.5	170	480	3,750	-	5.0	-	0.1
B5	7.70	4850	3067	646	651	<0.1	52	141	788	4	<1	<1	794	137	1,150	0.6	-	-	<0.01
B6	7.85	5100	2970	1650	375	<0.1	235	260	430	5	<0.1	3.3	455	46	1,550	0.2	7.6	-	<0.02

Table 3.3 Composition of groundwater samples analysed relative to seawater. The equivalent ion concentration in diluted seawater (DSW) is calculated from the samples conductivity value. Measures of DSW hardness, alkalinity, calcium, sodium, potassium, sulphate and chloride are expressed as mg/L. The relative seawater balance (RSB) of individual ions and measures of hardness and alkalinity are expressed as the percentage (%) present in each sample relative to the DSW concentration. Sample SWC refers to a seawater control.

		Hardness		Alkalinity		Calcium		Magnesium		Sodium		Potassium		Sulphate		Chloride	
Sample	**Conductivity**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**	**DSW**	**RSB**
SWC	**52400**	6480	100	318	100	409	100	1320	100	10500	100	398	100	2780	100	19080	100
LV1	46700	5775	63	283	59	365	78	1176	60	9358	99	355	18	2478	59	17005	93
LV2	14430	1784	78	88	1033	113	100	364	73	2892	88	110	0	766	39	5254	79
LV3	2690	333	322	16	1286	21	462	68	295	539	32	20	18	143	6	979	65
LV4	13300	1645	78	81	193	104	136	335	66	2665	87	101	12	706	51	4843	89
SE1	5710	706	79	35	118	45	139	144	68	1144	87	43	13	303	6	2079	85
SE2	3580	443	199	22	1335	28	501	90	144	717	67	27	5	190	37	1304	54
SE3	15570	1925	88	94	778	122	196	392	67	3120	85	118	0	826	33	5669	81
DD1	3870	479	77	23	617	30	152	97	63	775	89	29	19	205	48	1409	71
DD2	14100	1744	191	86	646	110	483	355	137	2825	69	107	21	748	89	5134	88
DD3	10650	1317	134	65	2491	83	242	268	112	2134	80	81	27	565	110	3878	58
SB1	7630	944	8	46	3736	60	22	192	2	1529	135	58	41	405	0	2778	72
SW1	2170	268	41	13	197	17	242	55	2	435	92	16	19	115	96	790	54
CW1	3350	414	15	20	516	26	46	84	9	671	98	25	18	178	10	1220	75
CW2	1720	213	21	10	6668	13	112	43	5	345	114	13	153	91	7	626	26
CW3	14000	1731	31	85	75	109	156	353	8	2805	108	106	10	743	1	5098	95
CW4	3740	463	61	23	291	29	267	94	21	749	89	28	38	198	5	1362	77
NW1	3920	485	223	24	1581	31	631	99	147	785	56	30	37	208	162	1427	66
B1	4540	561	220	28	1757	35	468	114	173	910	55	34	3	241	46	1653	74
B2	20000	2473	182	121	107	156	433	504	135	4008	67	152	19	1061	100	7282	89
B3	9700	1200	71	59	1736	76	110	244	65	1944	104	74	3	515	35	3532	81
B4	10500	1298	93	64	223	82	171	265	79	2104	102	80	76	557	86	3823	98
B5	4850	600	108	29	2212	38	138	122	115	972	81	37	10	257	53	1766	65
B6	5100	631	262	31	1212	40	590	128	202	1022	42	39	12	271	17	1857	83

As suggested by Boyd (2002), conductance (µS/cm) was employed as the standardised measurement because of its accuracy at low salinities. Salinity (ppt) was calculated for all samples by converting conductivity using a multiplication factor of 0.00063.

3.3 Results

The varied geographical and geological nature of the water provided a wide range of results for most elements.

3.3.1 pH

The pH of all groundwater samples ranged between 8.6 (CW1) and 6.8 (B2).

3.3.2 Conductivity/salinity.

Conductivity of the samples ranged between 46,700 and 1,720µS/cm (29.9 to 1.1ppt). The Lockyer Valley sample (LV1) had the highest conductivity of 46,700µS/cm and a sample from the central west (CW2) had the lowest 1,720µS/cm. The average conductivity for all samples was 9,644µS/cm (6.1ppt).

3.3.3 Hardness

Hardness ranged from 4,494mg/L (B2) to 45mg/L (CW2). The 6 samples from western Queensland (SB1, SW1, CW1, CW2, CW3 and CW4) that were deficient in magnesium had the lowest RSB hardness values of 61% or less. Eight other samples (LV1, LV2, LV4, SE1, SE3, DD1, B3 and B4) had RSB values between 63% and 100%. The remaining samples had RSB values of 100% or more (LV3, SE2, DD2, DD3, NW1, B1, B2, B5 and B6).

3.3.4 Alkalinity

Primarily a response of the strong presence of bicarbonates, groundwater RSB values for alkalinity were high with the exception of CW3. This sample had an RSB value of 75%. Alkalinity ranged from 1,730 mg/L (SB1) to as low as 26mg/L (SW1).

3.3.5 Sodium

Sodium ion (Na^+) levels ranged between 9,825 mg/L (LV1) to just 170 mg/L (LV3). Of the 23 samples tested, 15 had Na^+ levels within ± 25% of their DSW values while a further 6 were within ± 50% of their DSW value. Two samples, LV3 and B6, had the lowest RSB values of 32% and 42% respectively. Both samples were relatively high in calcium and magnesium. Only one sample (SB1), had Na^+ levels significantly above its DSW value with an RSB value of 135%. Given the high alkalinity of this sample, the Na^+ present in SB1 would be in the form of sodium bicarbonate.

3.3.6 Magnesium.

Magnesium (Mg^{2+}) levels ranged from as high as 710mg/L (LV1) to as low as 1mg/L (SW1). Eight samples (LV1, LV2, LV4, SE1, SE3, DD1, B3 and B4) had Mg^{2+} levels of between 60 and 100% of their DSW value. A further eight samples (LV3, SE2, DD2, DD3, NW1, B1, B3, B5 and B6) had between 112 and 295% of their DSW value. Six samples from western Queensland SB1, SW1, CW1, CW2, CW3 and CW4, had the lowest Mg^{2+} levels with respective RSB values of 2, 2, 9, 5, 8 and 21%.

3.3.7 Calcium

Calcium (Ca^{2+}) levels ranged from 676mg/L (B2) to 12 mg/L (CW1). Only three samples had RSB values for Ca^{2+} that were less than 100% (LV1, SB1 and CW1). The other 20 samples had more Ca^{2+} than predicted by their respective DSW values. Seven samples (LV3, SE2, DD2, NW1, B1, B2 and B6) had Ca^{2+} levels that were at least 400% of the DSW value. NW1 had the highest relative Ca^{2+} content with an RSB value of 631%.

3.3.8 Potassium.

Potassium (K^+) ranged from not detectable (<1mg/L) to as high as 63mg/L (LV1). Potassium was generally found to be deficient in almost all groundwater samples with 21 of the 23 different samples having less than half of their RSB values. The exceptions were CW2 and B4 that had RSB values of 153 and 76% respectively.

3.3.9 Chloride

Chloride (Cl^-) ions ranged from as high as 15,810mg/L (LV1) to as low as 164mg/L (CW2). Of all 23 samples, 13 had Cl^- RSB values between 75 and 100% (LV1, LV2, LV4, SE1, SE3, DD2, CW1, CW3, CW4, B2, B3, B4 and B6). Nine samples (LV3, SE2, DD1, DD3, SB1, SW1, NW1, B1 and B5) had RSB values of between 54 and 75%. Sample CW2 had the lowest relative Cl^- content with an RSB value of just 26%.

3.3.10 Sulphate

Sulphate (SO_4^-) levels ranged from not detectable (<1mg/L) to 1,450mg/L (LV1). Only 3 samples had RSB values equal to (B2) or higher (DD3 and NW1) than 100%. Six samples had RSB values between 51 and 100% (LV1, LV4, DD2, SW1, B4 and B5), 7 were between 17 and 48% (LV2, SE2, SE3, DD1, B1, B3 and B6) and 7 had RSB values of 10% or less (LV3, SE1, SB1, CW1, CW2, CW3, and CW4).

3.3.11 Other Ions

Fluoride levels were low (≤1) in all but 2 samples (SB1 and CW2). Nitrate levels above 1mg/L were observed in 6 samples (SE2, CW4, NW1, B1, B4, and B6). Where measured, phosphate and iron levels were typically low.

3.4 Discussion

Globally, the majority of inland prawn farming is conducted at salinities of less than 5ppt (Boyd and Thunjai, 2002). In this study the water samples tested ranged between 1,720 and 46,700µS/cm (1.1 and 29.9ppt) with an average of 9,644µS/cm (6.1ppt). Data from over 1,700 bores studied in the Burdekin region (refer to previous chapter) show that around 40% deliver water with conductivities of between 1,720 and 20,000µS/cm. Less that 5% of bores are over 20,000µS/cm while the remaining 55% have recorded maximum conductivities of less than 1,720µS/cm. The black tiger prawn (*P. monodon*) has been reported to grow in water with conductivities reaching as low as 250µS/cm (0.16ppt) (Saha et al, 1999). However, the successful production of marine prawns inland is not simply a question of identifying the right salinity.

Groundwater must have the appropriate ratios of major cations and anions to be suitable for prawn culture. Boyd et al (2002) recommended the ratios of major ions should be similar to those found in seawater. Significant mortalities of prawn PL have been observed in water with high or low cation to anion ratios (McGraw and Scarpa, 2004). As seen in this study, some sources of groundwater can have chemistries quite different to that of seawater. An exception to the general rule proposed by Boyd et al (2002) may occur when the presence or absence of a particular ion becomes beneficial. An example of this may be the high calcium levels observed in groundwater in this study. High calcium and bicarbonate levels are favoured in pond aquaculture of crustaceans. Calcium is required during moulting and bicarbonate buffers the pond pH. An example of a beneficial deficiency is the absence of sulphate. Low sulphate levels in groundwater would have positive pond health and management implications. Sulphate is the source of hydrogen sulphide in anaerobic pond soils (Boyd, 2003).

As in seawater, the conductivity of groundwater is primarily influenced by the presence of sodium (Na^+) and chloride (Cl^-) ions. However unlike seawater, the relative contributions of these two ions to the total dissolved ion (TDI) value of groundwater can be quite variable. In seawater the major ion is Cl^-. In this study Na^+ was the major ion in two groundwater samples (SB1 and CW2). In both samples this appears to be the result of an excess of bicarbonate (HCO_3). This excess of HCO_3, most likely in the form of sodium bicarbonate ($NaHCO_3$), is responsible for the high level of alkalinity observed in both samples, and to an extent, the proportionally low contribution by Cl^-. In the low salinity sample (CW2), HCO_3 accounted for almost 48% of the TDI. This is extremely high compared to the 1% contribution HCO_3 made to TDI in the seawater sample. Similarly in SB1, the contribution by HCO_3 to TDI approaches 28%. This is almost equal to that of Cl^- (32%) in this sample. By contrast in seawater, Cl^- accounted for almost 55% of the TDI. In other samples the Cl^- concentration was almost 4 times that of the Na^+ concentration (LV3 and B6). In these samples the concentrations of other cations, specifically calcium (Ca^{2+}) and magnesium (Mg^{2+}), were the highest observed in all of the samples tested relative to their conductivity. These samples also recorded the highest relative hardness values of all groundwater samples tested.

Hardness is influenced directly by the presence of Ca^{2+} and Mg^{2+} ions. Calcium levels in tested waters were above their comparative seawater values (DSW) in all but three of the samples tested. These exceptions (LV1, SB1 and CW1) were not confined to a single region or water type. However, Mg^{2+} levels in artesian waters from central Queensland were without exception low (SB1, CW1, CW2, CW3, CW4). As a result, the hardness of samples from this region were low compared to seawater and were the lowest observed in this study.

Adequate Ca^{2+} levels are necessary to permit the formation of a prawn's new exoskeleton during the course of each moult cycle (McGraw and Scarpa, 2003). Unlike freshwater crustaceans such as redclaw which store calcium for this purpose (Huner et al., 1979), marine prawns draw the calcium directly from the water. At low salinity, marine prawns may not have enough calcium to achieve proper shell hardening. Boyd and Thunjai (2003) proposed that minimum levels of calcium for proper shell hardening would approach 30mg/L. This minimum calcium requirement would be satisfied by all but the central western region water samples. Waters deficient in Ca^{2+} can be supplemented through the addition of agricultural grade gypsum (calcium sulphate, $CaSo_4$). To raise calcium levels by 30 mg/L an application of gypsum would cost approximately AU$35 per ML.

The largest and most consistent variation between the concentration of ions in seawater and inland waters was observed for potassium (K^+). In only two cases, a sample from the Burdekin region (B4) and the central west (CW2), were the K^+ levels within 50% of that present in seawater at equivalent salinity. The result for this central western sample may be explained in part by its low conductivity. The Burdekin sample, which had over 60mg/L K^+ or 76% of its DSW concentration, demonstrates how localised geological factors can influence individual ion concentrations and produce aquifers that are more suitable for inland prawn culture than others in the same region.

Generally, the water chemistry results are similar to those observed in inland water samples from most states in the USA where groundwater is used for inland prawn farming. Generally, such as in Arizona, Alabama, Texas and Florida, potash (KCl) is added to alleviate deficiencies in potassium. The exact dose and frequency of additions is dependent on the water chemistry and the rate of water exchange or loss. Data from other countries such as China, Thailand and Ecuador show that such practices are not always necessary. Potassium levels of groundwater in these countries tend to be similar to that of seawater at the same salinity (Boyd and Thunjai, 2003). In Australia, addressing potassium levels deficiencies in saline groundwater has been identified as a necessary practice to enable the use of this water for production of marine finfish (Fielder, 2001).

To address the imbalance in potassium content of groundwater, muriate of potash (KCl) can be added. Potash dissolves readily and for every 1kg/ML added, the K^+ concentration would increase by 0.5mg/L. Therefore to treat a sample like SE2 with a conductivity of 3,580µS/cm and a K^+ concentration of 1mg/L, approximately 50kg of potash per ML would be required. This would restore K^+ levels to those normally found in seawater at this salinity (27mg/L) at a cost of AU$33/ML.

The availability of groundwater with appropriate chemistries will be a major factor in determining the prospects for individual farms, as well as the development of inland marine prawns industry as a whole. The ability of species such as the black tiger prawn to grow at satisfactory rates even at salinities considered 'fresh', provides this industry with great scope in Queensland. Again, using the example of the Burdekin delta, if black tiger prawns can be grown successfully at salinities of 800 µS/cm or greater (>0.5ppt), then 70% of the bore water in the area could be utilised for aquaculture of this species.

While the analyses of groundwater in this study was limited, it demonstrates that regions like the Burdekin, Darling Downs and others possess good quality groundwater, with favourable chemistries likely to be suited to the production of marine prawns. However, a simple analysis of water chemistry does not necessarily provide enough information to determine if it is suitable for prawn culture. It is recommended that water chemistry, including major cations, anions, pH, hardness and alkalinity be conducted as the first step in assessing the suitability of an individual bore for aquaculture. These tests should then be followed by specific and carefully controlled bioassays using prawn postlarvae already acclimated to the appropriate salinity. Once these preliminary tests are completed more in depth analysis of water chemistry may still be required before investing in inland prawn culture.

4. Animal Supply and Husbandry

4.1 Prawn Postlarvae

Black tiger prawns (*P. monodon*) were sourced from commercial hatcheries at 15-18 days of age (post-hatch). These prawn postlarvae (PL) were either transferred into 200L acclimation bins for the purpose of small-scale laboratory trials or placed into 5,000L holding tanks so they could be acclimated to salinities approaching that of growout ponds.

Banana prawn (*P. merguiensis*) juveniles (<0.5g) were sourced from the Bribie Island Aquaculture Research Centre (BIARC). These animals were stocked directly into 200L tanks for the purpose of assessing the salinity tolerance of this species.

4.2 Prawn Postlarvae Holding Facilities

Postlarvae were purchased from a local hatchery and kept in two 60m^3 outdoor tanks for 16 days before the experiments started. During this period, prawns were fed with commercial starter pellets (37 % crude protein; Charoen Pokphand, Thailand) twice a day at 09:00 and 16:00. Temperature, salinity and pH in the holding tanks varied between 23.4 – 25.6°C, 33.7 – 34.1ppt and 7.9 – 8.5, respectively. All tanks were aerated to maintain adequate dissolved oxygen.

4.3 Adult Prawns

Adult *P. monodon* (20 – 25g) were obtained from a commercial prawn farm by cast netting. These animals were maintained at a density of less than 5 animals per square meter in 1,000L fibreglass tanks and inspected for any external injury or signs of stress. Animals were fed a commercial pellet twice daily.

5. Acclimation Trials

5.1 Objectives

The purpose of these trials was to determine the most suitable rate of acclimation for prawn postlarvae (PL) from a salinity of 34ppt to less than 2ppt. Two trials were conducted. The first involved establishing the most favourable rate of acclimation for black tiger prawns (*P. monodon*), while the second investigated the ability of juvenile banana prawns (*P. merguiensis*) to adapt to salinities as low as 5ppt using the same methods.

5.2 Methods

5.2.1 Penaeus monodon:

Three acclimation rates (3, 5 and 7 days) were assessed against a constant, full-strength seawater control. Each treatment was conducted in triplicate. The $PL_{15\text{-}18}$ were stocked in 30L fibreglass tanks at a rate of 50 individuals / tank. All tanks were initially filled with 5L of full-strength seawater and were continuously aerated. Salinity was gradually diluted from 34ppt to 1.7ppt by dilution with dechlorinated municipal water (0ppt) within 3, 5, or 7 days. The dilution rates were 0.44, 0.27 and 0.19ppt hr^{-1} respectively for the 3, 5 and 7 day treatments. The rate of dilution was controlled using a freshwater header tank located above each replicate tank.

On the last day of the acclimation period, the prawns from each treatment, and the water in which they had been acclimated, were transferred to individual 300L polyethylene tanks. These tanks held larger amounts of new water at the same salinity. Similar handling protocols were applied to the control tanks. Once the acclimation targets had been reached (salinity and timescale), survival of prawns was recorded at 1, 6, 24, 48 and 72hr intervals.

Prawns were fed with commercial pellets at a rate of approximately 5–10% of biomass per day throughout the entire trial. All tanks were cleaned daily and at least 50% of each tank's volume was exchanged daily using water of the same salinity and temperature. During the later stages of the acclimation process, the larger volumes of water added as part of the dilution procedure, negated the need for further water exchanges for the purpose of maintaining water quality.

5.2.2 Penaeus merguiensis:

This experiment was conducted to determine the ability of juvenile *P. merguiensis* (<0.5g average weight) to adapt from full strength seawater to salinities as low as 5ppt. Four target salinities were selected (15, 10, 7.5 and 5ppt) to establish the lowest favourable salinity range for this species. Acclimation was conducted over a period of 7 days (0.19ppt hr^{-1}) using the same stock split into three 300L polyethylene tanks. As per previous trials, dechlorinated municipal water (0ppt) provided the freshwater source. Upon reaching the first target salinity (15ppt) up to 20 animals were randomly selected from the 3 acclimation tanks and transferred to 30L fibreglass tanks. Subsequent samples were progressively removed as target salinities were achieved. The pattern of mortality for PL at each target salinity was monitored at 24hr intervals for a period of up to 96 hrs.

5.2.3 Statistical methods:

The time-series nature of the data was taken into account by an analysis of repeated measures for each trial, via AREPMEASURES of GenStat (2000). This forms an approximate split-plot analysis of variance (split for time) for percentage survival data. These were followed by theoretically-correct individual within-time analyses of binary counts, using the generalized linear model analysis of GenStat (2002), with a Binomial distribution and logit link function (McCullagh and Nelder, 1989).

5.3 Results

5.3.1 Penaeus monodon:

During acclimation no significant differences ($P>0.05$) in mortality were observed (Table 5.1). However, post-acclimation mortality was significantly higher ($P>0.05$) in 3 and 5 day treatments. The 72hr post-acclimation mortality totalled 25.3 ± 5.49% and 30.0 ± 5.78% respectively in the 3 and 5 day acclimation treatments. This level of mortality was significantly lower ($P<0.05$) than in the control and 7 day acclimation treatment. Although the mean level of mortality was higher in the 5 day treatment, the patterns of post-acclimation mortality for 3 and 5 day acclimation treatments were similar ($P>0.05$).

Table 5.1 Cumulative mortality (%) of *Penaeus monodon* postlarvae at 3,5 and 7 day acclimation rates from 34ppt to 1.7ppt. Data are means ± standard error.

Cumulative mortality (%)	Acclimation rate			
	Control	3 day	5 day	7 day
During acclimation	2.0 ± 1.98	1.3 ± 1.62	2.0 ± 1.98	3.3 ± 2.54
24 hrs post-acclimation	2.0 ± 1.94	18.7 ± 5.41 [a]	24.7 ± 5.98 [a]	4.0 ± 2.72
72 hrs post-acclimation	4.7 ± 2.66	25.3 ± 5.49 [a]	30.0 ± 5.78 [a]	4.0 ± 2.47

[a] Within rows, means are significantly different from control values ($P<0.05$).

5.3.2 Penaeus merguiensis:

Survival of *P. merguiensis* in salinities from 15 to 5ppt is presented in Table 5.2. Significant levels of mortality ($P<0.05$) were observed in 5 and 7.5ppt treatments. The highest total mortality (83.72 ± 5.21%) occurred in the 5ppt treatment at 96hrs. This was significantly higher than all other treatments ($P>0.05$). Mortality was significantly elevated above the 15ppt treatment in the 5 and 7.5ppt treatments after 24hrs ($P>0.05$).

Table 5.2. Cumulative post-acclimation mortality (%) of *Penaeus merguiensis* (<0.5g) acclimated from 34ppt to 15,10, 7.5 and 5ppt. Data are means ± standard error.

	Cumulative Post-acclimation Mortality (%)			
Salinity (ppt)	24hrs	48hrs	72hrs	96hrs
15	0	0	0	0
10	0	0	0	0
7.5	6.25 ± 3.24 [ab]	10.42 ± 4.08 [ab]	16.67± 4.98 [ab]	20.83 ± 5.43 [ab]
5	27.91 ± 6.33 [abc]	65.12 ± 6.73 [abc]	79.07 ± 5.74 [abc]	83.72 ± 5.21 [abc]

[a] Within columns, means are significantly different ($P<0.05$) from 15ppt.
[b] Within columns, means are significantly different ($P<0.05$) from 10ppt.
[c] Within columns, means are significantly different ($P<0.05$) from 7.5ppt.

5.4 Discussion

Many authors have noted that following a period of acclimation, black tiger prawns (*P. monodon*) can be successfully farmed at low salinity (Shivappa and Hambery, 1997; Limsuwan, 2000; Szuster and Flaherty, 2000; Althitan et al., 2001; Boyd, 2003). Successful acclimation of marine crustaceans to low salinities must provide enough time for the balance of sodium and chloride ions in the animal's hemolymph to reach equilibrium. In order to maintain their osmotic balance, prawns will reduce sodium and chloride passage across the gills as well as water loss from the gut (Navas and Sebastian, 1989). The time required for adult *P. monodon* to stabilise hemolymph osmolality has been shown to be 48hrs (Diwan et al, 1989). If the osmotic pressure becomes too low, as can be the case if the period allowed for acclimation is too short, then the prawn will die. In this study, the slowest acclimation rate yielded the lowest rate of mortality for *P. monodon* PL_{15-18} adapted to a salinity of 1.7ppt.

Most penaeid prawns adapt readily to low salinities as juveniles but as adults they are more sensitive to salinity change (Dall, 1981). The best age to acclimate PL to low salinity environments is when the animal's gill development is complete, (Valencia, 1976). Usually this occurs 15 days after hatch. Attempting to acclimate PL too early or too late can result in significant mortality. Pantastico and Oliveros, (1980) clearly demonstrated that *P. monodon* postlarvae (PL_{20}) did not acclimate to freshwater as readily as slightly older animals (PL_{35}). Conversely, older animals (PL_{90}) did not cope as well as younger animals (PL_{35}). Studies with other species such as *L. vannamei* also demonstrate that the ability to cope with acclimation to low salinities is dictated by PL age and stage of development (McGraw et al., 2002). In this study, all *P. monodon* acclimation trials were conducted with PL that were less than 35 days old.

Successful acclimation of *P. monodon* PL to low salinities can also be influenced by the rate at which this acclimation is conducted. Zhang et al. (1989) demonstrated that *P. monodon* survival varies enormously depending on the rate of acclimation. In the present study, survival of *P. monodon* PL appeared unaffected by the rate of dilution at the completion of the acclimation period (Table 5.1). Our results agree with Pantastico and Oliveros (1980), who observed that survival of *P. monodon* PL_{35} was high (98-99%) at salinities between 0 and2ppt when acclimated over a period of three days. However, studies such as Pantastico and Oliveros (1980) and others (Valencia, 1976; Gaudy and Sloane, 1981; Cawthorne et al., 1983) did not assess the consequences of acclimation rate on long-term survival. The elevated levels of mortality observed in the present study for more rapid acclimation treatments, suggests that short-term estimates of survival may not accurately reflect the long-term consequences of acclimation.

In this study, PL were challenged with salinity changes ranging from full strength seawater (34ppt) to those approaching freshwater (1.7ppt). Prawn PL may be better able to cope with the acclimation procedures used in this study if already adapted to more brackish salinities. Alternatively, even slower rates of acclimation may improve survival at low salinities and adaptation to freshwater environments. It is also advisable to use the pond water during the acclimation phase to lower salinity in order to reduce the physiological stress of adjusting to different water chemistries during stocking.

Inland prawn farmers in Thailand typically access 12 - 15 day old *P. monodon* PL which have already been adapted to 10ppt water over a period of 3 – 5 days (Szuster and Flaherty, 2000). Further acclimation is conducted on farm using a variety of methods which include slowly mixing pond water with transport water and using separate nursery facilities (Miller et al., 1999). The most common method however, involves building an earthen bund or PVC pen in a corner of the growout pond into which the PL are placed (Szuster and Flaherty, 2000). This allows farmers to partially fill the growout pond with freshwater which is then used to gradually dilute the higher salinity water contained within the bund/net pen area over a period of 7-10 days. During the course of growout, salinities may reach as low as 0ppt. At such low salinities, growers usually add mineral supplements to improve moulting and growth (Boyd et al., 2002).

Athithan et al. (2001) also successfully demonstrated that *P. monodon* (PL_{35}) could be gradually acclimated from seawater (23ppt) to freshwater at a rate of 5ppt/week (16 days). Studies have also demonstrated that once acclimated, *P. monodon* not only survive, but grow well in fresh and low salinity waters in India (Rajyalaksham and Chandra, 1987; Guru et al., 1993; Saha et al., 1999).

The poor survival observed for juvenile *P. merguiensis* in response to attempts to acclimate them to salinities below 10ppt may indicate that this species is not as tolerant to low salinities. These results are consistent with those published by Zacharia and Kakati (2002) who also found that survival of *P. merguiensis* PL was poor at 5ppt. Growth trials conducted using juvenile *P. merguiensis* by Zacharia and Kakati (2002) demonstrated that while this species returned better survival rates at higher salinities (25 and 35ppt), the best growth response was observed at 15ppt. Reasons for this superior growth at brackish salinities was proposed by Zacharia and Kakati (2002) to be related to the life history of this species. Alternatively, the poor survival observed in this study may indicate that the animals used were too old and like *P. monodon, P. merguiensis* must be acclimated at a much earlier age. This species spends most of its postlarval and juvenile life within brackish estuarine environments. Further studies on the acclimation of *P. merguiensis* to salinities below 10ppt and the resultant growth performance are therefore required.

If acclimation strategies can be developed for *P. merguiensis,* this species has some biological attributes that would make them attractive for inland prawn farming operations. The ability to reproduce this species using domesticated broodstock may provide opportunity for the development of inland hatcheries and selection of more 'freshwater tolerant' strains. In addition, the omnivorous nature of *P. merguiensis* may suit more high density 'heterotrophic' systems if it can be demonstrated that they are able to feed upon the flocs of bacteria and other organic matter generated by these systems.

The biological advantages and disadvantages of both *P. monodon and P. merguiensis* will have to be weighed by proponents against the type of system being developed, its location and mode of operation.

The results of these trials demonstrate that appropriate care must be taken when acclimatising these prawn species to low salinity. Important factors such as the age of PL, the salinity at the start of acclimation, the final target salinity, and the conditions under which acclimation occurs, must all be considered when devising an acclimation strategy.

6. Bioassays

6.1 Objectives.

To assess the suitability of individual groundwater types for prawn aquaculture, a series of controlled bioassays using were conducted. The same assays were also used to determine if any deficiency in potassium levels in groundwater could be addressed through the addition of mineral supplements.

6.2 Methods

6.2.1 Bioassays

These trials were carried out over a period of approximately two years using multiple batches of black tiger prawn (*P. monodon*) PL. Groundwater samples were collected from a number of regions in Queensland (Table 6.1) using 60L HDPE screw top barrels. One groundwater sample from the Tiaro area (SE1) was repeated with two different batches of PL.

Table 6.1. Region, locality and sample names of water used in bioassays.

Region	Locality	Sample	No of bioassays
South-East	Lockyer Valley	LV1, LV2, LV3, LV4	1 for all samples
	Tiaro	SE1, SE2, SE3	2 for SE1; 1 for SE2 and SE3
Southern	Darling Downs	DD1, DD2, DD3	1 for all samples
South-West	Surat Basin	SB1	1
	Mitchell	SW1	1
Central-West	Longreach	CW1, CW2, CW3, CW4	1 for all samples
North-West	Mt Isa	NW1	1
North Coastal	Burdekin	B1, B2, B3, B4, B5, B6	1 for all samples

Three treatments were used to assess the suitability of individual water samples. The primary treatment involved the addition of potassium (K^+), in the form of potassium chloride (KCl), to levels that would be present in seawater at the same salinity. Survival of PL in this treated sample (TGW) was compared to that of PL held in a sample of the same water that did not receive the K^+ supplementation – untreated groundwater (UGW). The third treatment was a seawater control (SWC) that was diluted with dechlorinated municipal water (0ppt) to the same salinity as the groundwater treatment.

Prawn $PL_{15\text{-}18}$ were stocked into 300L polyethelene tanks filled with 100L of seawater at 34ppt and 23.5°C and continuously aerated. The salinity of the acclimation tanks was gradually diluted from 34ppt to as low as 1ppt at a rate equivalent to 0.19ppt/hr. A single batch acclimation was typically used as the source of PL for all treatments. As the target salinity for each treatment was reached, PL were transferred into individual 2L plastic bowls using a fine meshed scoop net (Figure 6.1).

Approximately 20 PL were counted into each bowl. Each bowl was supplied with gentle aeration and PL were fed a measured amount of prawn starter feed daily until completion of each trial. Uneaten food and faeces was removed from the bowls daily. Mortalities were counted and removed at 24, 48 and 72hrs. At the completion of the assay (72hrs) survivors were also recorded in order to account for cannibalism that can occur following moulting.

Figure 6.1. Experimental bioassay set up. Each assay was conducted in triplicate 2L plastic bowls supplied with continuous gentle aeration and a measured amount of feed daily. Uneaten feed and faeces was removed.

In all experiments temperature was at ambient levels (22 – 25°C). Photoperiod was set at 14 hrs light and 10 hrs dark.

6.2.2 Statistical analysis

Experimental data, expressed as percent survival, were analysed by Genstat (6.1 for Windows) using generalized linear model (i.e. modelling of binomial proportions by logits) (Payne et al. 1993; McCullar & Nelder 1983).

6.3 Results

The results of bioassays conducted in this study clearly demonstrate the effectiveness of potassium addition in improving survival of *P. monodon* PL in saline groundwater. The ionic profile of water samples used for bioassays is given previously (Table 3.2).

6.3.1 Observations of survival in individual water samples.

LV1: A significant difference in survival was observed between the UGW group (100% mortality at 24 hours) and the SWC and TGW groups at 24, 48 and 72hrs (Figure 6.2). The respective 72hr survivals for SWC and TGW groups of 88.3 ± 3.6% and 90 ± 3.4% were not significantly different from each other.

LV2: Only 1.6 ± 2.6% of the prawns in the UGW group survived to 24hrs (Figure 6.3). No further mortality occurred in the UGW group past this point. Survival in the TGW group fell to 68.3 ± 9.9% at 24hrs to 18.3 ± 6.7% after 72 hours. These results differ significantly from the 100% survival observed after 72hrs for the SWC group.

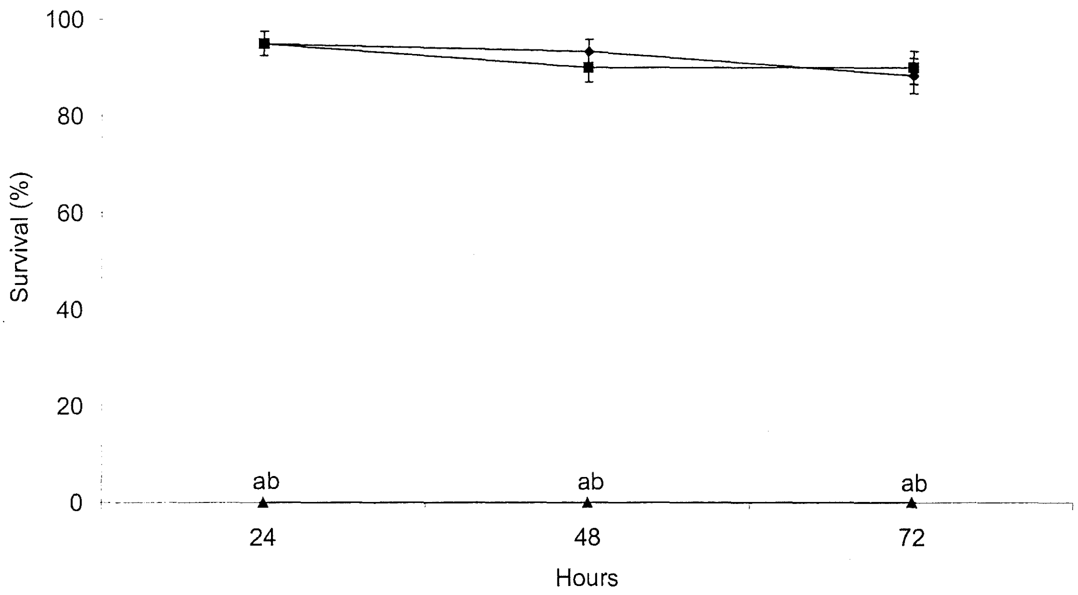


Figure 6.2 LV1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control and b Indicates the treated groundwater value is significantly different (P<0.05) from untreated groundwater.

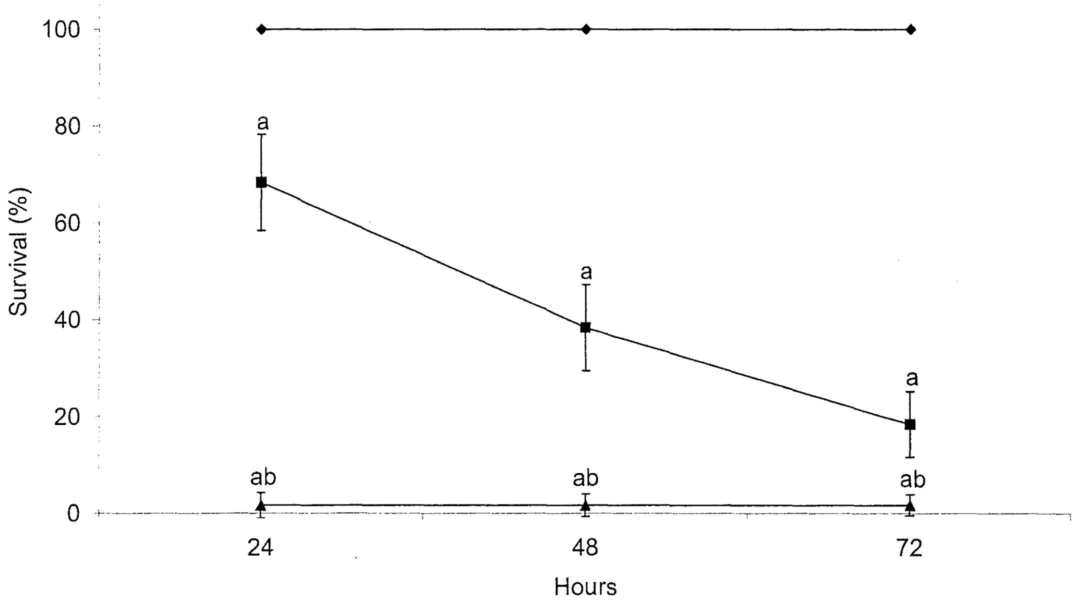


Figure 6.3 LV2. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

LV3: In this sample 100% mortality was observed in the UGW group within the first 24hrs (Figure 6.4). Significant mortality was also recorded in SWC and TGW groups with only 30 ± 9.7% and 11.7 ± 6.8% respectively surviving the first 24hrs. Survival continued to decrease in the SWC and TGW groups with the final 72hr survival being 16.7 ± 6.5% and 6.7 ± 4.4% respectively. The only significant differences in the final survival rates were found to exist between SWC and UGW groups.

LV4: At 24hrs 100% mortality had occurred in the UGW group (Figure 6.5). Survival in the TGW group was recorded as 86.7 ± 4.3% at this point and fell slightly to 81.7 ± 4.9% at 48hrs and no further mortality occurred in this group. No mortality was observed in the SWC group throughout the duration of the trial.

SE1: (a) At 24hrs survival was high in all treatments (Figure 6.6). However in the UGW group, survival fell significantly to 71.4 ± 0.1% by 48hrs. By 72hrs, survival in the UGW group had fallen further to 49 ± 9.7%. This survival was significantly lower than both the SWC (88.9 ± 5.6%) and TGW (100%) groups. There were no significant differences in survival between TGW and SWC groups. (b) Survival rates across all three treatments were very similar until the 72-hour observation period. At 72hrs survival in SWC and TGW groups was 53.3 ± 5.6% and 55 ± 5.6% respectively (Figure 6.7). The survival of the UGW group at 72hrs was significantly less than the SWC and TGW groups with just 28.3 ± 5.1% of animals alive.

SE2: Although generally low, survival was significantly higher in the SWC group (45 ± 10.6%) at 24hrs than the UGW group (16.7 ± 7.9%) (Figure 6.8). Survival in the TGW group at 24hrs was 31.7 ± 9.9%. Differences between TGW and UGW groups became significant after 48hrs when mortality reached 100% in UGW group and 25 ± 7.9% in the TGW group. No further mortality occurred in the TGW group to 72hrs. At 72hrs, survival in the SWC group was also low (20 ± 7.0%) but not significantly different from the TGW group.

SE3: There was no significant difference between the high survival of both SWC (100%) and TGW (98.3 ± 1.6%) groups at 72 hrs. In contrast UGW, which had only (6.7 ± 3.1%) at 24hrs, had suffered 100% mortality by 48hrs (Figure 6.9).

DD1: In this assay the SWC and TGW groups displayed similar patterns of mortality (Figure 6.10). Survival in the SWC group fell to 70 ± 5.3% by 24hrs and was 56.7 ± 5.6% by 72hrs. These values were not significantly different from the 71.7 ± 5.2% and 63.3 ± 4.8% in the TGW at 24 and 72hrs. In UGW, significant differences were observed at all time points with 50 ± 5.8% surviving 24hrs and 31.7 ± 5.2% 72hrs.

DD2: In contrast to the similar high survival rates of the SWC (91.7 ± 3.4%) and TGW (91.8 ± 2.7%) groups at 72hrs, the UGW group suffered 100% mortality within the first 24hrs (Figure 6.11).

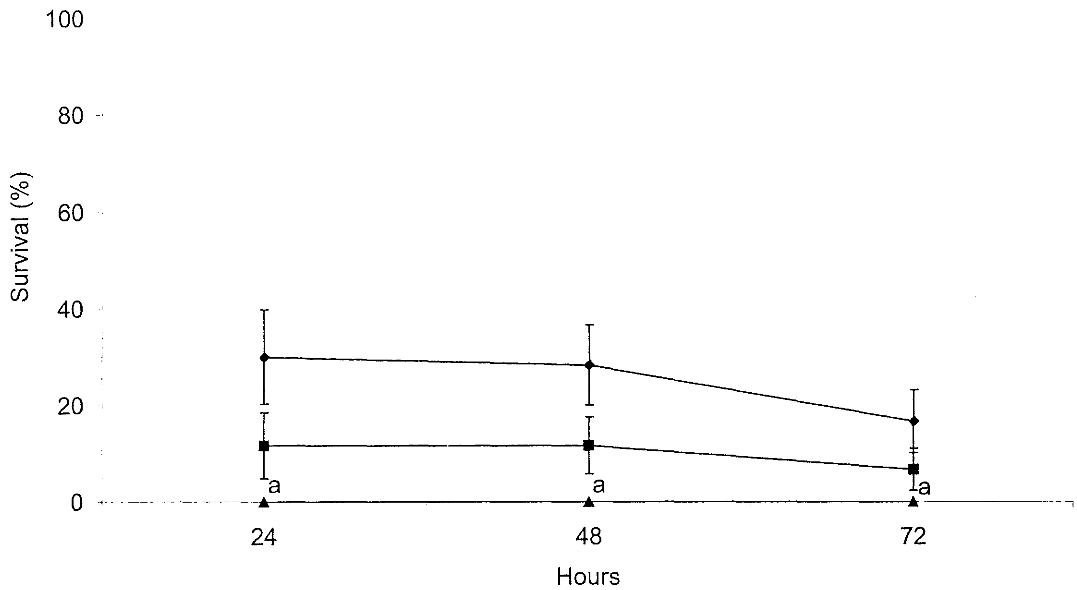


Figure 6.4. LV3. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

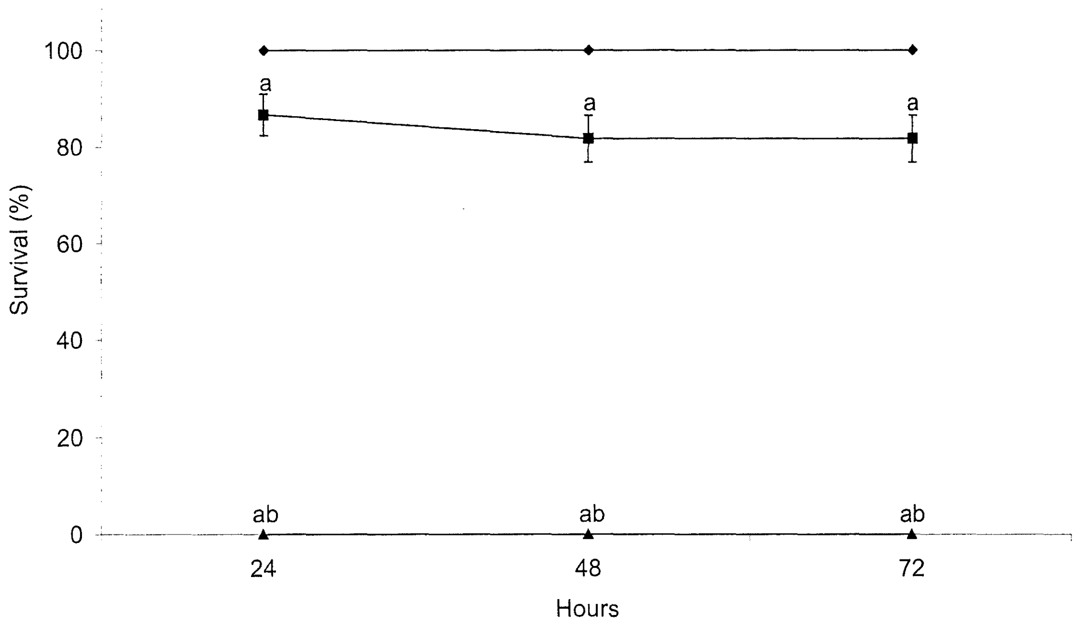


Figure 6.5. LV4. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

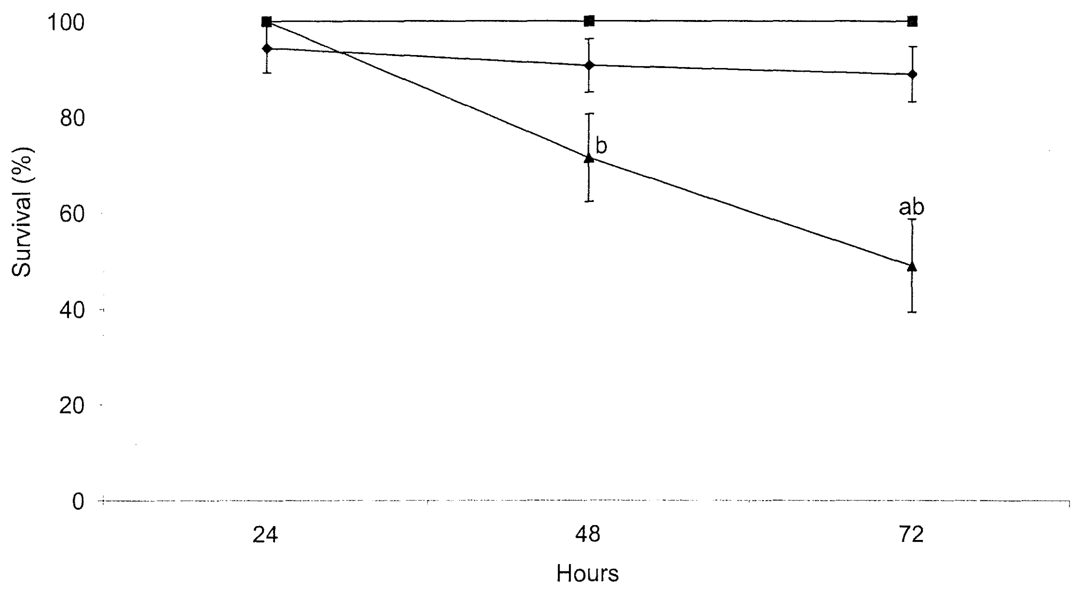


Figure 6.6. SE1(a). Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

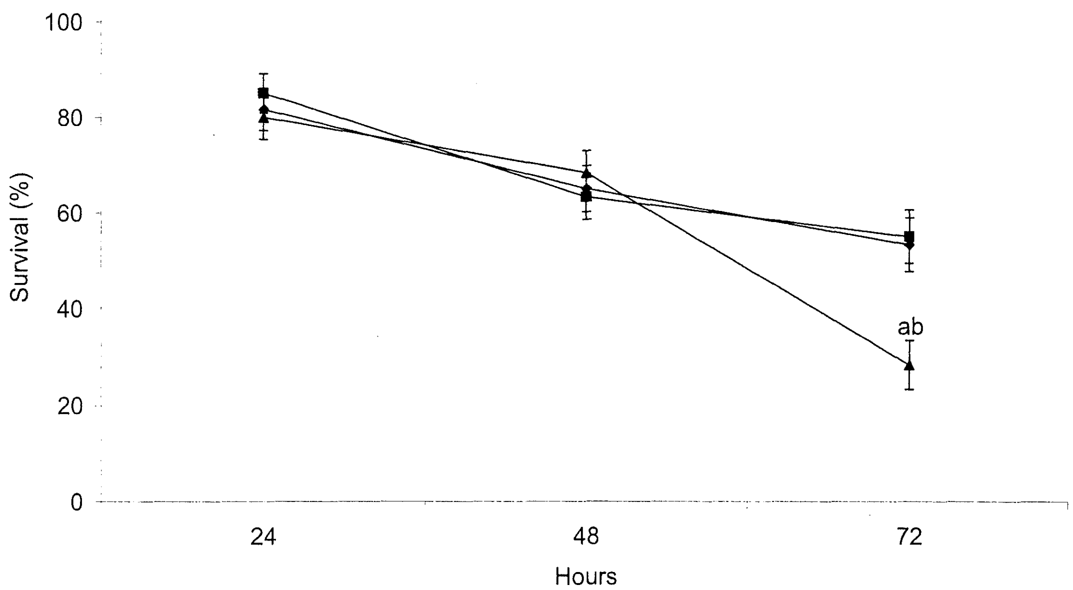


Figure 6.7. SE1(b). Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

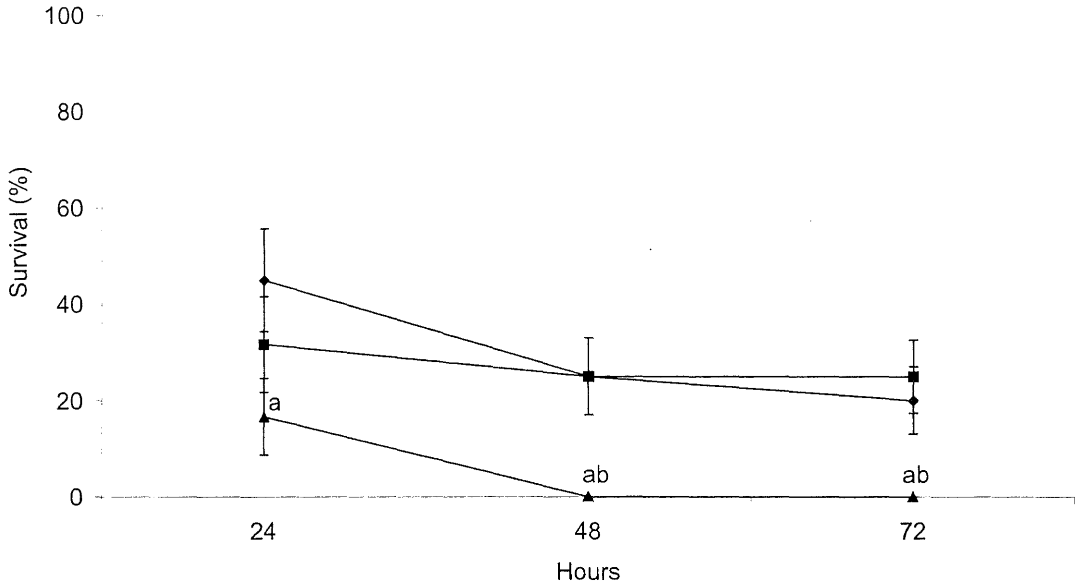


Figure 6.8. SE2. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

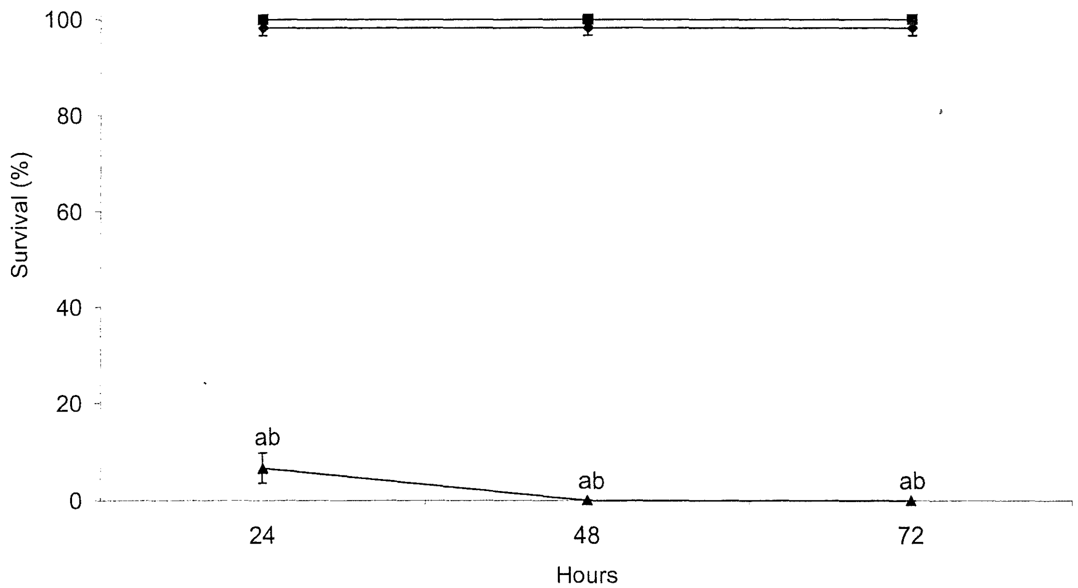


Figure 6.9. SE3 Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

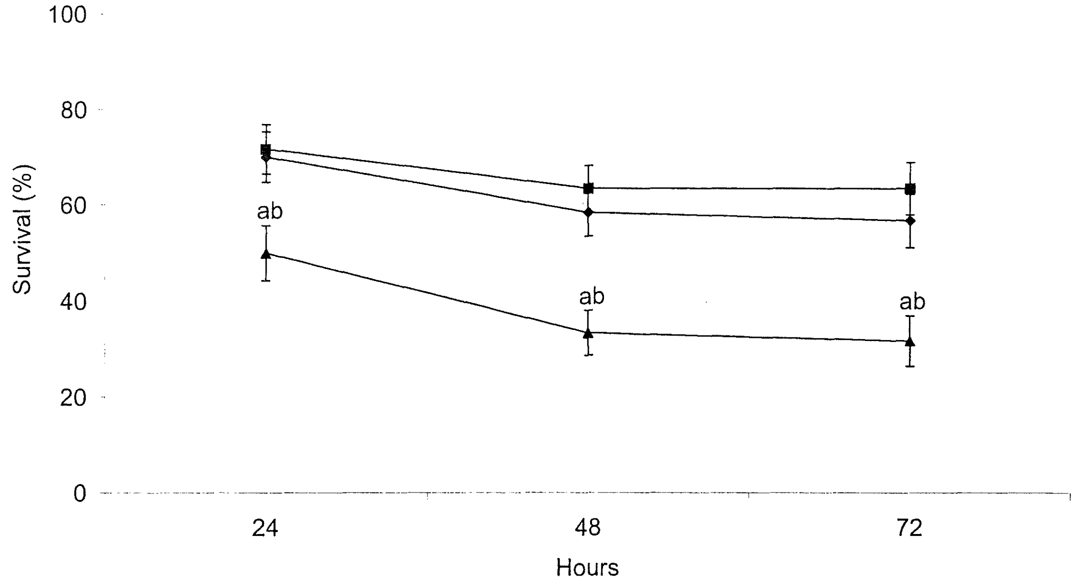


Figure 6.10. DD1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (♦) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

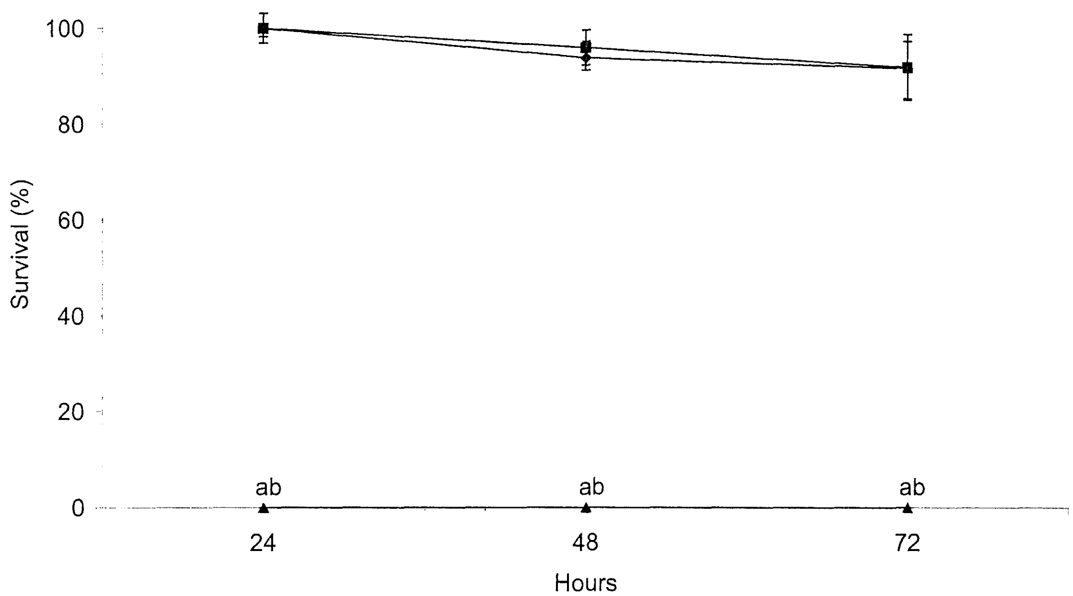


Figure 6.11. DD2. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (♦) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

DD3: Survival in UGW was significantly lower than in the SWC and TGW groups (Figure 6.12). The UGW group had a 72hr survival of 37.3 ± 9.3%. The SWC group had a survival of 87 ± 2.5% and the TGW group had an 82.8 ± 3.6% survival rate. There was no significant difference in survival between SWC and TGW groups.

SB1: In contrast to the SWC group at 24hrs (100% survival) complete mortality was observed in both TGW and UGW groups (Figure 6.13). Survival in the SWC group decreased to 62 ± 9.5% at the completion of the assay (72hrs).

SW1: A significant difference was observed in survival at 24hrs between the SWC group (20 ± 8.5%) and both the TGW (82.5 ± 8.3%) and UGW (80 ± 8.9%) groups (Figure 6.14). This pattern remained consistent throughout the assay. Survival at 72hrs was 20 ± 6.2% for the SWC group, 56.1 ± 8.9% for TGW and 45.5 ± 9.1% for UGW groups. No significant differences in survival between TGW and UGW groups were observed in this assay.

CW1: At 24 hours survival in the SWC group was 31.8 ± 9.4% (Figure 6.15). By 72hrs survival in this group averaged 13.6 ± 5.7%. These figure were not statistically different to those of the UGW group. At 24hrs UGW survival was 28.3 ± 9.6% and by 72hrs this had fallen to 3.3 ± 3.1%. Although survival in the TGW group fell to 50 ± 10.6% at 24hrs, the survival rate of 38.3 ± 8.5% at 72hrs was significantly higher than the SWC and UGW groups.

CW2: In TGW and UGW groups 100% mortality occurred within 24hrs (Figure 6.16). Although 25 ± 5.2% survival was recorded in SWC at 24 hours, 100% mortality was also observed in this group at 48hrs.

CW3: Survival in both the SWC (97.8 ± 3%) and TGW (98 ± 2.6%) groups was significantly higher than in the UGW group at 72hrs (Figure 6.17). In the UGW group mortality was complete within 48hrs.

CW4: At 24hrs survival in the SWC group (75 ± 9.1%) was significantly less than in TGW (98.3 ± 2.7%) but not UGW (95 ± 4.6%) (Figure 6.18). However, by 72hrs survival in the SWC, TGW and UGW groups (55 ± 8.7%, 66.7 ± 8.6 and 70 ± 8.4% respectively) was not significantly different.

NW1: At 24hrs survival in TGW and UGW groups was high, 91.1 ± 3.5% and 92.2 ± 3.5% respectively (Figure 6.19). However, survival had fallen significantly by the end of the assay to 50 ± 4.5% in TGW and 54.9 ± 5.8% in UGW groups. In contrast, survival in the SWC group (84.5 ± 6.6%) at 72hrs was significantly better than either the TGW or UGW groups. Reduced survival in TGW and UGW may be explained by inadequate aeration of the sample prior to the start of the assay. Fine precipitate was observed on the inside of each TGW and UGW replicate which is indicative of calcium carbonate precipitation.

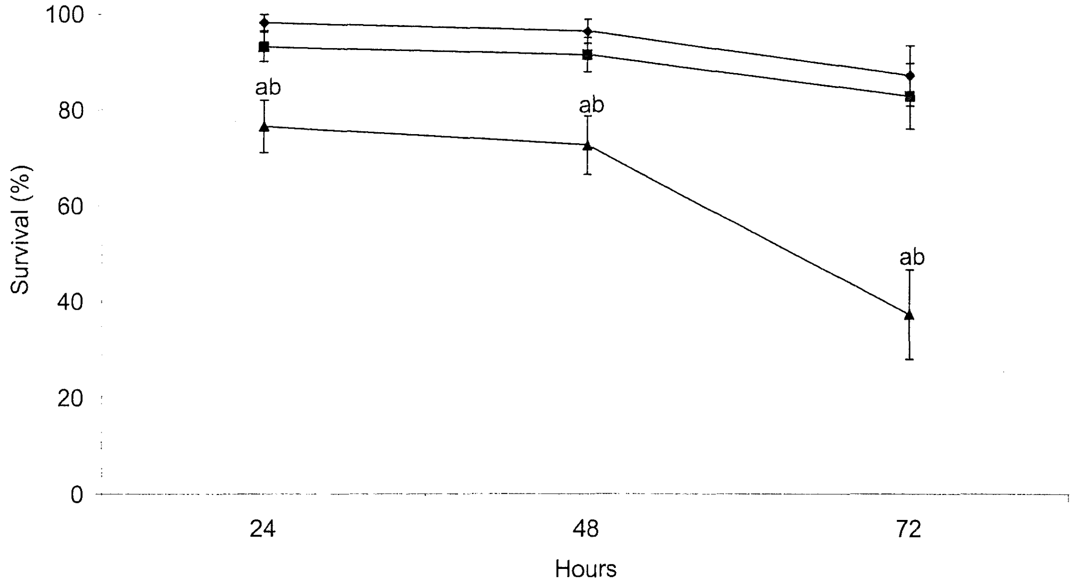


Figure 6.12. DD3. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

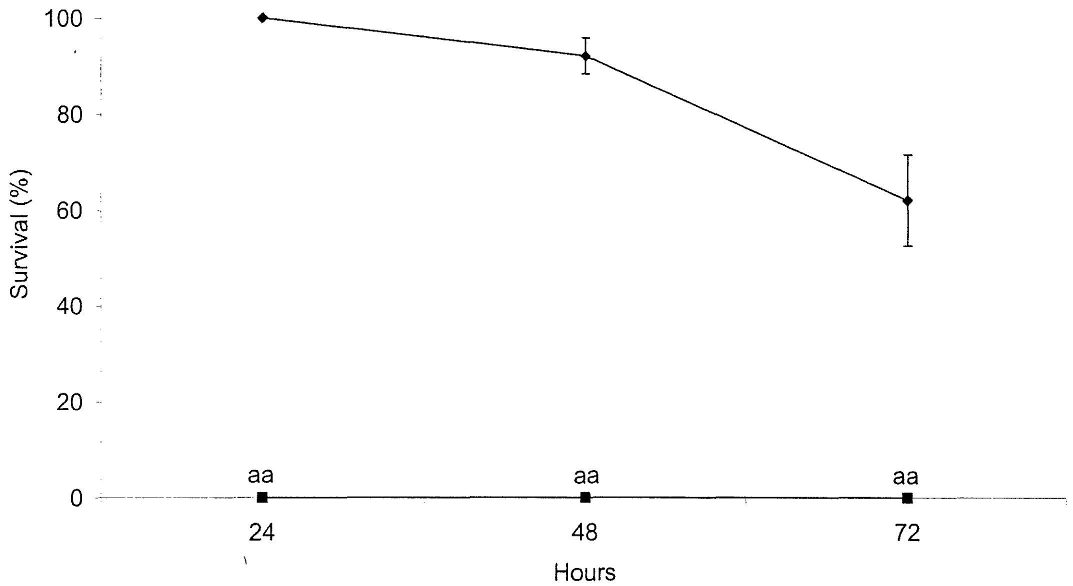


Figure 6.13. SB1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

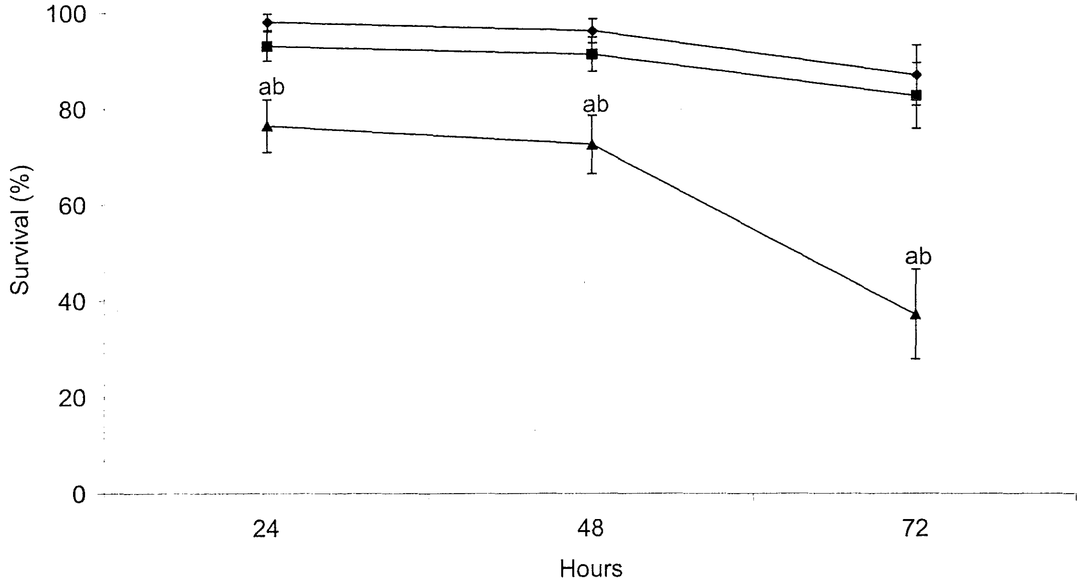


Figure 6.12. DD3. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (♦) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

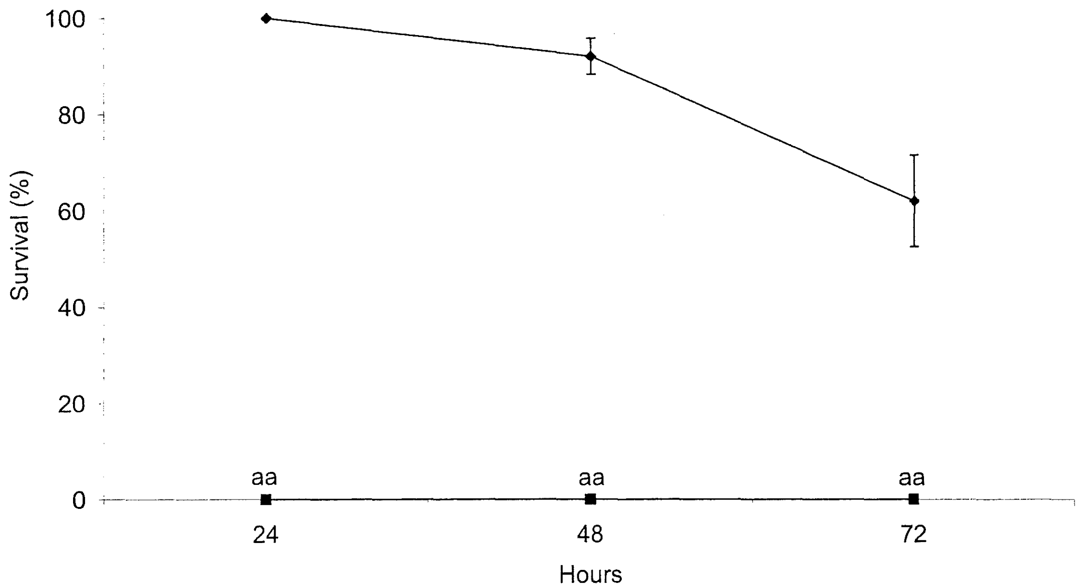


Figure 6.13. SB1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (♦) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

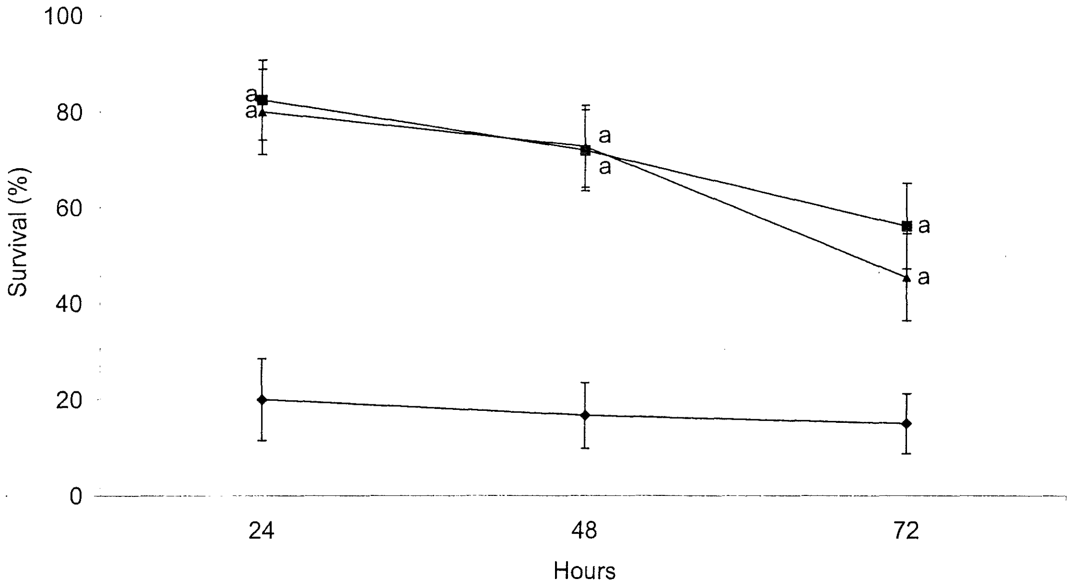


Figure 6.14. SW1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

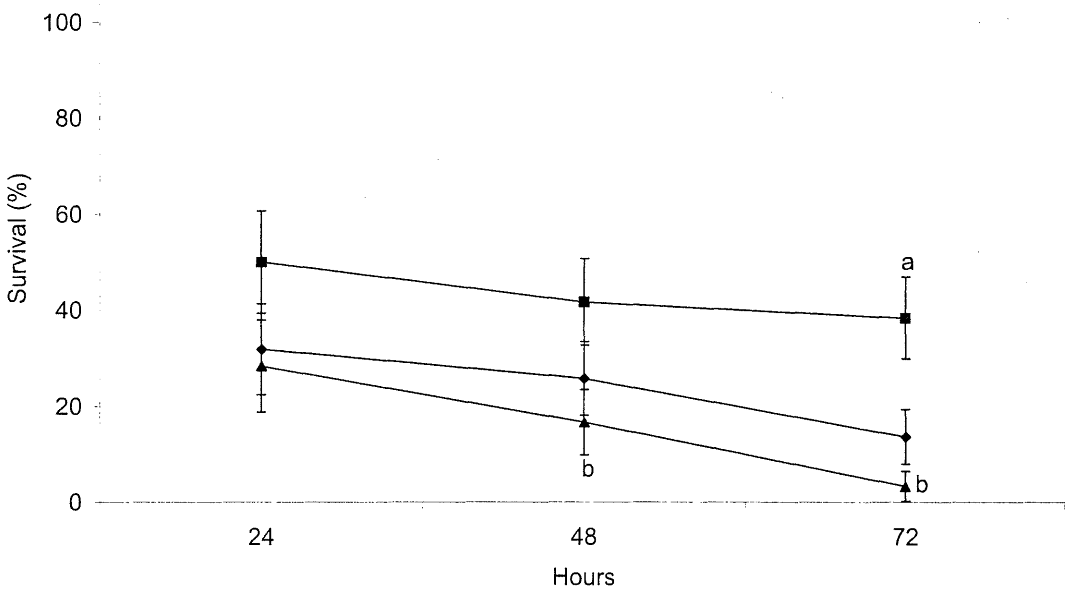


Figure 6.15. CW1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

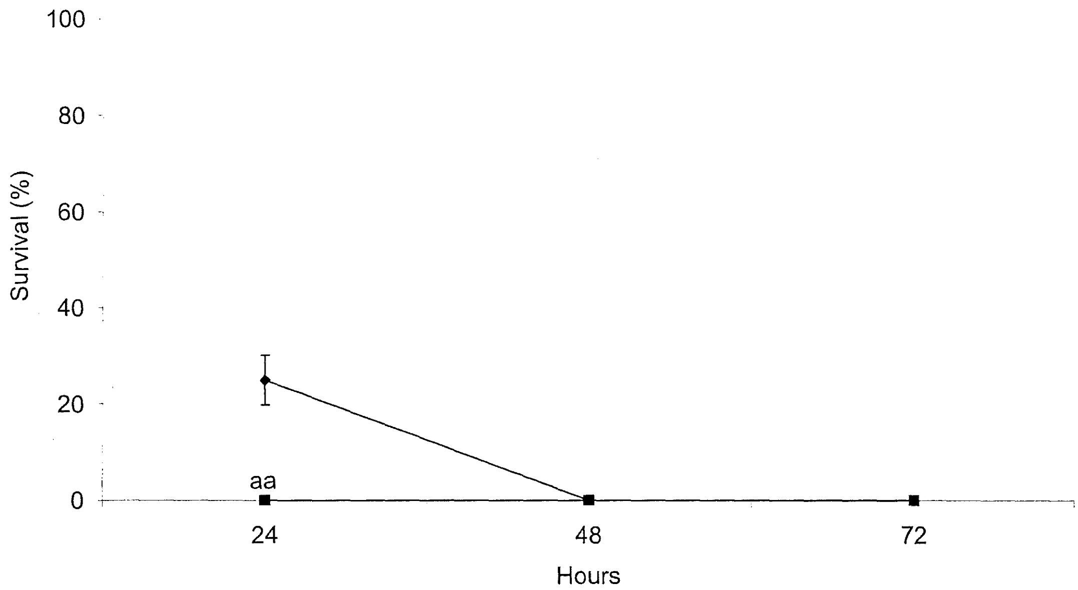


Figure 6.16. CW2. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

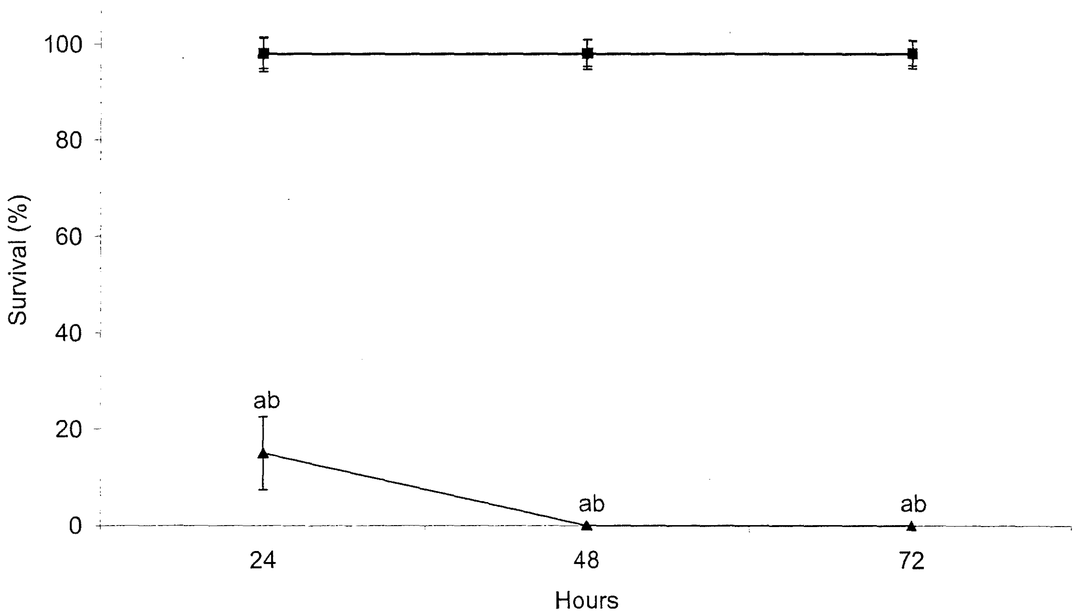


Figure 6.17. CW3. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

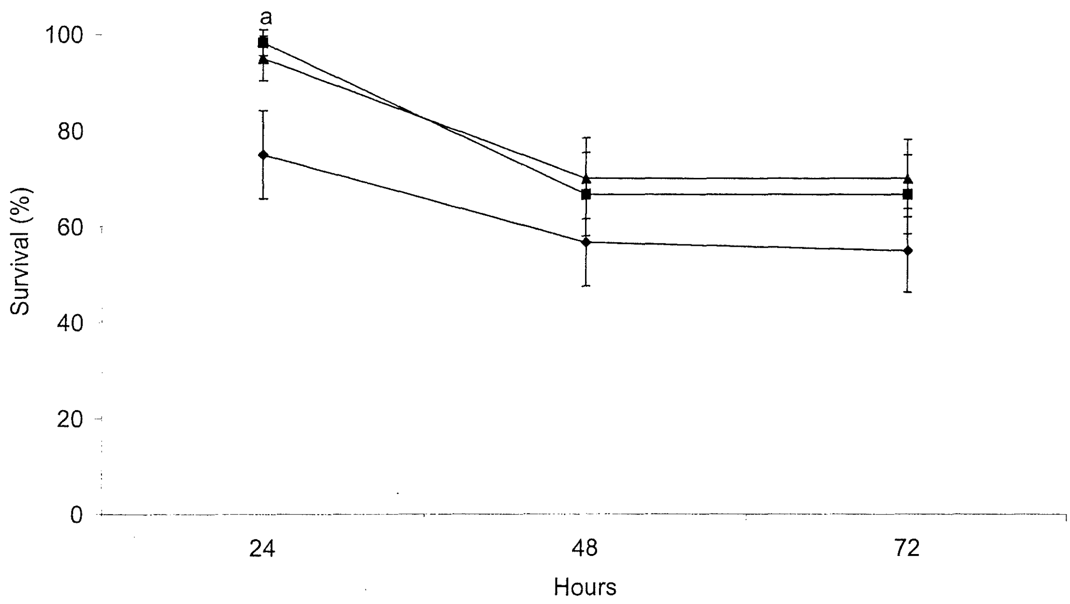


Figure 6.18. CW4. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

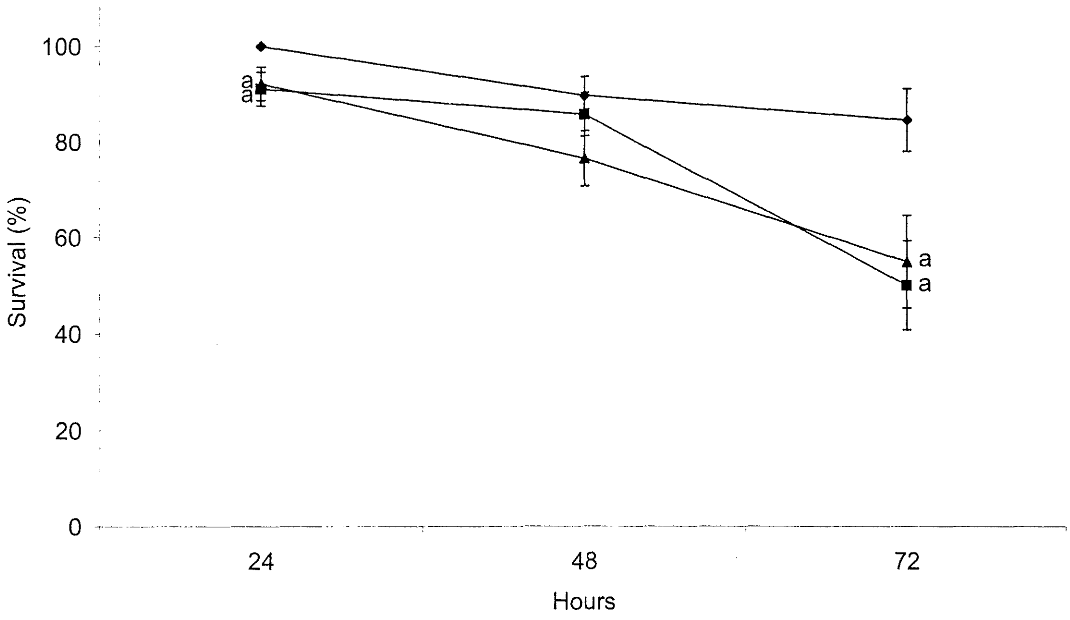


Figure 6.19. NW1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

B1: In this assay both the SWC and TGW groups had similar survival rates of 85 ± 4.1% and 88.3 ± 3.7% respectively (Figure 6.20). The final survival at 72 hrs of 68.3 ± 5.2% and 76.7 ± 4.8% for the SWC and TGW groups were not significantly different. However, the UGW group had significantly lower results with 68.3 ± 5.4% surviving to 24 hours and 45 ± 5.6% surviving to 72 hours.

B2: In the UGW group 100% mortality was observed within the first 24hrs (Figure 6.21). At this point survival was high in SWC and TGW groups, both at 96.7 ± 2.1%. Although there was a significant disparity between SWC and TGW groups at 48hrs, differences in the 72hr survival rates (83.3 ± 4.2% for SWC and 71.7 ± 5.1% for TGW) were not statistically significant.

B3: Mortality was 100% in the UGW group at 24hrs (Figure 6.22). Although the TGW group had 20 ± 4.6% survival at this time, only 1.6 ± 1.3% survival was observed in this group by 48hrs. Survival in the SWC group was significantly better with 86.7 ± 3.8% of animals making it to 72hrs. As in the case NW1 the low survival in this sample may be related to inadequate aeration of the sample prior to the start of the assay. Substantial carbonate precipitate formed on the inside of TGW and UGW bowls during the assay.

B4: No statistical difference was found between any of the treatments during this trial. At the 72hrs the SWC, TGW and UGW groups had survival rates averaging 75 ± 4.9%, 78.3 ± 4.6% and 76.7 ± 4.8% respectively (Figure 6.23). This sample was the only example tested of groundwater that had potassium levels approaching those of diluted seawater.

B5: Survival in the UGW group was significantly poorer than in SWC and TGW groups (Figure 6.24). Survival of 26.8 ± 6% in the UGW group at 24hrs fell to 0% by the 72hrs. At 24hrs survival in the SWC and TGW groups was 56.6 ± 6.9% and 50.8 ± 6.2% respectively. Survival in both the SWC and TGW groups at 72 hrs of 45.3 ± 6.8% and 47.7 ± 6.2% respectively, did not differ significantly from 24 or 48hr survival levels.

B6: No significant difference in survival rates between groups were observed in the first 24hrs (Figure 6.25). At 48hrs survival in UGW animals fell to 33.3 ± 7.6%. This was significantly lower than survival in the SWC (62.5 ± 6.8%) and TGW (72.7 ± 6.5%) at that time. At 72hrs only 5.6 ± 3.8% of UGW animals had survived, compared to 54.2 ± 7.2% and 70.5 ± 6.9% of prawns in the SWC and TGW groups. No significant difference was observed in survival rates of SWC and TGW groups.

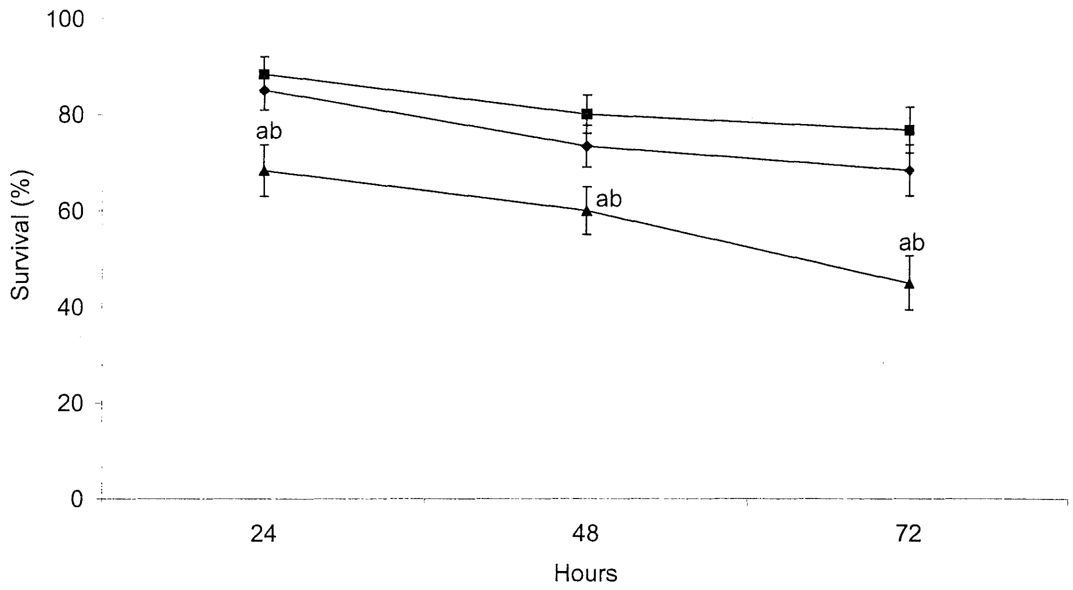


Figure 6.20. B1. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time

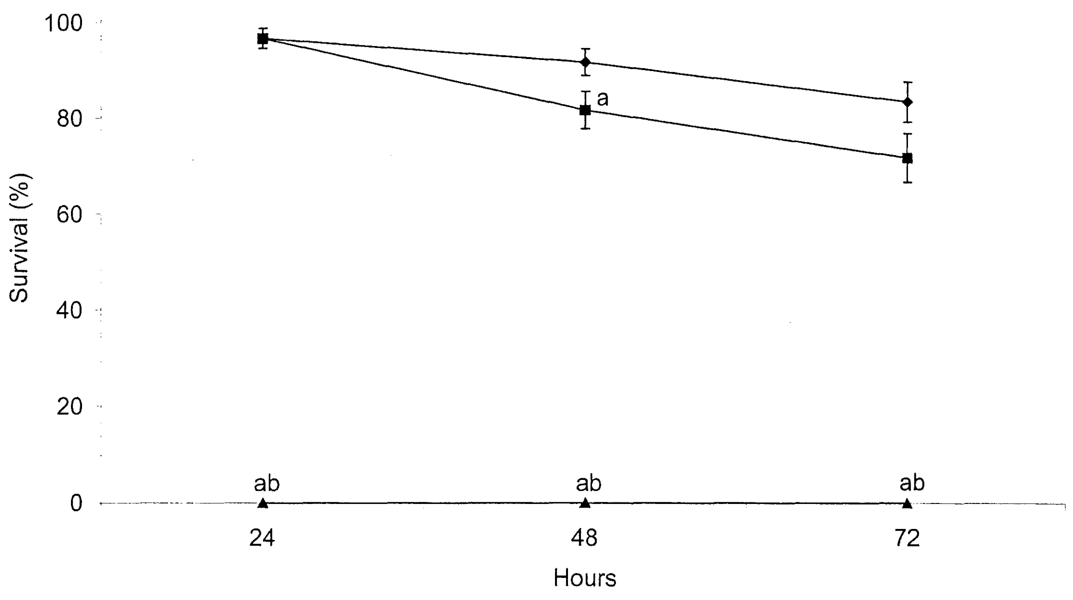


Figure 6.21. B2. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

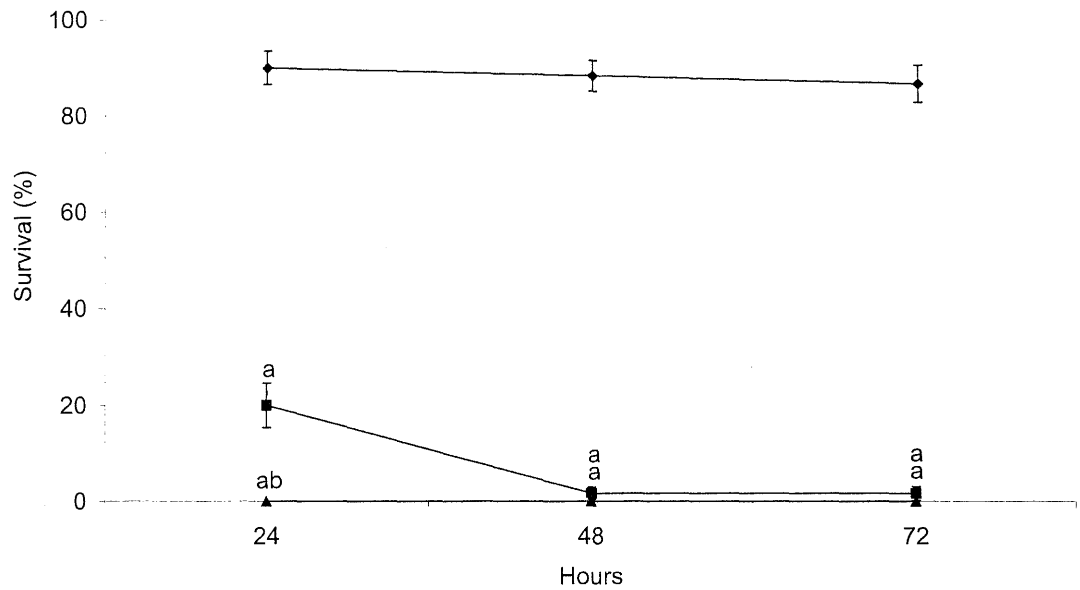


Figure 6.22. B3. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

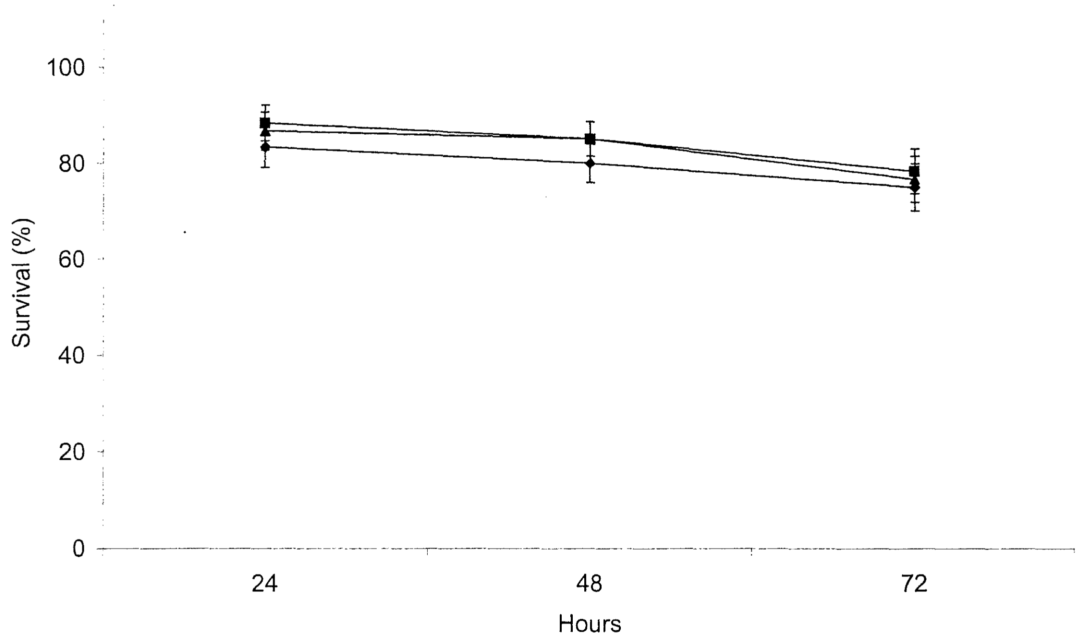


Figure 6.23. B4. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different (P<0.05) to the seawater control at that time, and b Indicates untreated groundwater is significantly different (P<0.05) from treated groundwater at that time.

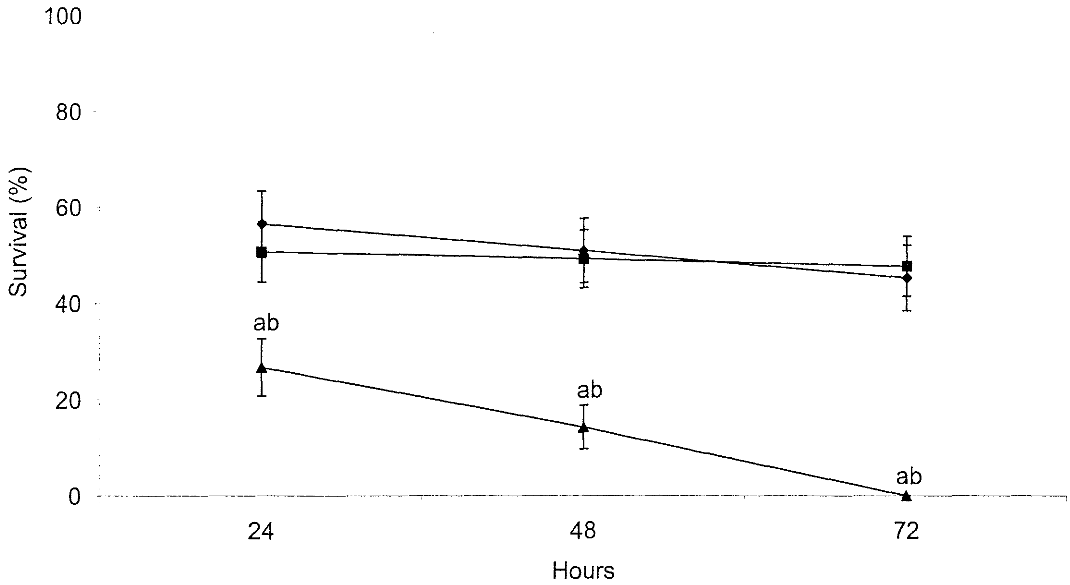


Figure 6.24. B5. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

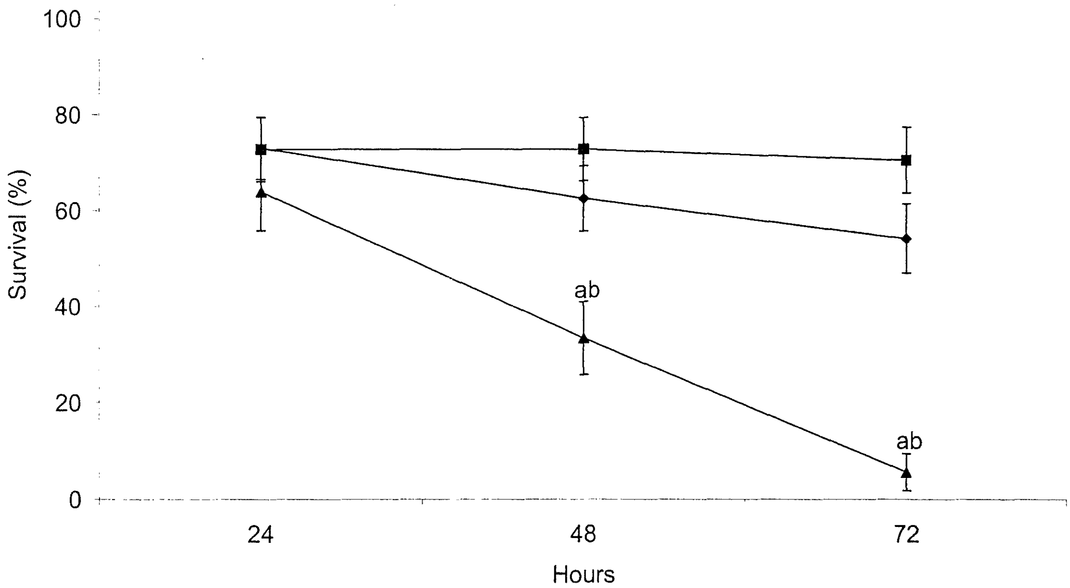


Figure 6.25. B6. Comparison of prawn PL survival held in treated groundwater (■), untreated groundwater (▲) and seawater control (◆) water at 24 hr intervals. All values are given as mean ± standard error. a Indicates the value is significantly different ($P<0.05$) to the seawater control at that time, and b Indicates untreated groundwater is significantly different ($P<0.05$) from treated groundwater at that time.

6.4 Discussion

The results of these bioassays demonstrate that not only are some water types more suitable than others, but that in most cases the addition of potassium chloride (KCl) will improve survival significantly. The data also demonstrates that individual bores, even those considered close in regional terms, can have quite different prospects with respect to their suitability for prawn survival and therefore aquaculture.

In this study, potassium (K^+) levels were restored to the equivalent of seawater diluted to the same salinity. Hemolymph K^+ in *P. monodon* is strongly regulated and shows less variability in response to reduced salinity than other major ions like sodium and chloride (Lin et al., 2000). The results from this study indicate that if K^+ levels are below one third of the relative seawater concentration then significant and acute mortality will occur. In three samples, one from the Central West (CW4), another from the North West (NW1) and the third from the Burdekin (B4), the addition of KCl did not significantly improve survival. In the case of the CW4 sample, the sample's K^+ level approached 38% of that present in seawater at the same salinity, and no significant differences were found in survival between the treated or untreated samples or the seawater control. Similarly, no significant differences were observed in the survival of PL in the NW1 sample where K^+ levels approached 37% of the SWC. The only sample where K^+ levels were close to that of diluted seawater was the Burdekin sample (B4). In this case the K^+ concentration was 76% that of seawater. The addition of the remaining 24% of K^+ did not significantly improve survival.

Supplementation of K^+ deficient waters with agricultural grade potash (KCl) is a simple and cost effective means of addressing any deficiency of this ion. The addition of mineral supplements including potash has also been shown to significantly improve survival in white shrimp (*L. vannamei*) reared in ponds filled with K^+ deficient groundwater in Alabama, USA (McNevin et al., 2004). The exact dose and frequency of additions is dependent on the water chemistry and the rate of water exchange or loss. At higher salinities, or in large volume flow-through systems, the cost of this treatment and the physical means of its application will have to be carefully considered. Even in static systems high rainfall events have the potential to increase the need for mineral supplementation to counter the effects of excessive dilution.

The poor survival of control animals in some assays demonstrates that the survival of prawns in groundwater can also be dependent on factors other than just variations in water chemistry. Salinity, the quality of prawn PL and handling stress are all factors that might influence survival during a bioassay. Post-acclimation mortality may be more significant in poorer quality PL especially at lower salinities. The response of PL to a salinity stress test, which measures the response of a batch of PL to low salinity water, is commonly used as a measure of PL fitness (quality). While no stress tests were used to determine PL quality within each experiment, the potential for variation in results between bioassays was evident when one sample (SE1) was repeated with a different batch of PL. Although the general pattern of mortality was similar, survival in both the groundwater and seawater replicates were up to 40% lower in the second assay (SE1(b)) compared to the original (SE1(a)). This result highlights that bioassays must be carefully controlled with their results considered in the context that variations commonly occur across all treatments and between different batches of PL. Variability between bioassays is likely to be most evident at lower salinities where the effects of acclimation stress and handling are most significant. Despite the potential for PL variability, our bioassays provided important preliminary information concerning the suitability of particular water types for prawn culture.

Survival was generally lower in bioassays that were conducted at low salinities (≤2ppt). The sample with the lowest conductivity (CW2) of 1,720µs/cm (1.1ppt) also returned the poorest survival for control animals (0%) from any of the assays. Moderately low rates of survival were also observed in samples with low conductivities from the central (CW1: 13.6 ± 5.7%) and south west (SW1: 20. ± 6.2%) regions. In both cases, survival in the treated groundwater samples was significantly better than in the control group. Survival at lower salinities is likely to be influenced by calcium (Ca^{2+}) levels. As stated previously, marine crustaceans are likely to have difficulty molting successfully in water when Ca^{2+} levels are less than 30mg/L (Boyd and Thunjai, 2003). However, the Ca^{2+} content of inland waters is often proportionally higher than in seawater. Of the 23 water types assayed, all but three had Ca^{2+} levels in excess of those present in their respective seawater controls. The relatively high Ca^{2+} levels found in low salinity groundwater may explain the improved survival of animals in some samples compared to their seawater control. In the case of SW1, with a conductivity of 2,170µs/cm, the calcium level was 2.42 times that found in diluted seawater. The Ca^{2+} content of 41mg/L in this sample is above the minimum 30mg/L required for molting success. Seawater at the same conductivity would have approximately 16mg/L of Ca^{2+}.

Early in the study it was observed that groundwater samples with elevated hardness and alkalinity levels needed heavy aeration prior to use. Otherwise, calcium carbonate ($CaCO_3$) would precipitate, foul the gills of PL, and cause significant mortalities. In a pond situation, water chemistry interactions are more complex through exchange of ions at the soil/water interface and in response to changes in pond water quality (e.g. pH). Therefore, the effects of high $CaCO_3$ levels in earthen ponds may not be as detrimental as those encountered in this study. The physical process of transferring water, filling ponds and topping up water levels are likely to assist in aerating the water and dropping out excess bicarbonate. However, it may be advisable to provide additional aeration of groundwater supplies to encourage precipitation of excess ions before it reaches the ponds. Aeration will also help degas groundwater that is saturated with carbon dioxide and nitrogen gases.

Survival at lower salinities can also be improved through the use of more specific acclimation strategies. As discussed in the previous chapter, more gradual methods that introduce the groundwater as part of the acclimation process, as opposed to when the target salinity is reached, are likely to yield significantly better survival at lower salinities. In order to improve on the survival observed in this study, individual acclimation tanks would be required for each water type. While not practical in a broad screen of samples, more site-specific acclimation and bioassay protocols are recommended where the intention is to employ a particular source of groundwater for the culture of a specific species.

Water from all regions tested provided samples with suitable characteristics for prawn farming. Positive bioassay results and the prevalence of high volume bores in regions such as the Darling Downs and the Burdekin delta suggest these areas would be suitable for development of inland prawn farms. The tropical climate of the Burdekin region would enable a longer growing season compared to the Darling Downs, providing more options with respect to the timing of stocking and the potential for multiple cropping.

Bioassays of this type provide an indication of the suitability of groundwater for aquaculture of marine crustaceans. They are a simple biological means of assessing the potential suitability of an individual groundwater type for prawn culture. Care should be taken in proceeding with any development if the water chemistry of the sample varies significantly from diluted seawater. While imbalances in major ions can potentially be corrected, the economics of such modifications must be carefully considered with respect to farm design, water use and operational constraints.

7. Growth Trials

7.1 Objectives

This trial was conducted to compare the short-term acclimation and survival of *P. monodon* PL in groundwater with their long-term survival and growth in this water. While a short-term bioassay will provide important information concerning the ability of PL to adapt to, and survive in groundwater, the long-term implications for growth are of primary concern for commercial development of inland prawn culture.

7.2 Methods

The waters used in this trial were selected on the basis of favourable bioassay results and their potential as sites for future open pond trials of inland prawn farming. These included Lockyer Valley (LV1), Tiaro (SE2) and Darling Downs (DD1) waters. The K^+ deficiencies identified in each of the water samples were addressed through the addition of KCl (Table 7.1).

Table 7.1. Potassium supplementation for Lockyer Valley (LV1), Tiaro (SE2) and Darling Downs (DD1) groundwater samples used in laboratory growth trials. All K^+ levels are expressed as mg/L.

Water	Salinity (ppt)	K^+ in water sample	K^+ in seawater at equivalent salinity	K^+ Added	Final K^+
LV1	29	63	354.71	291.71	354.71
SE2	2.8	5.70	43.37	37.67	43.37
DD1	1.8	5.60	29.39	23.79	29.39

Postlarvae (PL_{18}) *P. monodon* were collected from a 5,000L tank at BIARC in April 2000 with a daytime ambient water temperature of 23°C and salinity of 24.5ppt. Prawns were transferred with a soft net to two 300L tanks, where they were acclimated from 33.7 to 1.8ppt at a rate of 0.19ppt/hr using dechlorinated municipal water (0ppt). No mortality was observed during acclimation. A subsample of 20 PL was taken at the beginning of the acclimation (day 0) to determine the average starting length. These lengths were measured from the scale of the second antennae to the end of the telson using digital callipers.

Prawns were fed to satiation with Charoen Pokphand prawn feed (CP 4001-4002) during acclimation. Water level was adjusted as required during acclimation and after cleaning and siphoning. Once the acclimation tank reached the desired salinity (LV1 = 29ppt, SE2 = 2.8ppt and DD1 = 1.8ppt), prawns were transferred to 30L white fibreglass culture tanks.

Thirty PL per were placed into each tank. There were four replicate tanks for the treated groundwater treatments (TGW) and their respective seawater control (SWC). The SWC's were adjusted to the same salinity as the TGW samples using dechlorinated municipal water. The 30L fibreglass tanks were housed within a larger heated, circulating water bath to maintain temperature of all tanks at approximately 26°C. To reduce water exchange each tank was also fitted with a small aquarium filter. The filter intake was screened to prevent entrapment and damage to the PL.

Tanks were fed twice daily (morning and afternoon) and siphoned once a day. The siphoned water was passed through a 62μm filter screen and replaced into each tank. Ammonia concentrations were measured every two days and pH and dissolved oxygen measured weekly. The preset critical ammonia level for this experiment was 0.5ppm. Tanks that tested at or above this level were given a batch water exchange of up to 80% using water that had been adjusted to the appropriate salinity and water temperature.

On day 30 all the prawns were removed, blotted dry, counted, weighed and measured (Table 7.2).

Experimental data were analysed by Genstat (6.1 for Windows). Length and weight data were analyzed using a two-way ANOVA. Percentage survival data were analyzed using a generalized linear model (i.e. modeling of binomial proportions by logits). (Payne et al. 1993; McCullar & Nelder 1983).

7.3 Results

The average starting length for PL across all treatments was 13.35 ± 0.45 mm.

The average survival in TGW (40.00 ± 2.10%) was significantly higher than in SWC's (28.10 ± 1.90%) (Table 7.2). Within individual water treatments however, only the differences in survival for DD1 animals (TGW = 37.5 ± 3.70% and SWC = 20.00 ± 3.10%) was found to be significant.

Table 7.2. Mean survival (%), length (mm) and weight (mg) of prawn postlarvae reared in treated groundwater (TGW) of varying salinities from the Darling Downs (DD1), Tiaro (SE2) and Lockyer Valley (LV1) compared to seawater controls (SWC) of the same salinity for 30 days. Means by treatment and location ± standard error.

	DD1		SE2		LV1		Average	
	SWC	TGW	SWC	TGW	SWC	TGW	SWC	TGW
Survival (%)	20.0 ±3.1	37.5 ±3.7[a]	21.7 ±3.2	29.2 ±3.5	42.5 ±3.8	53.3 ±3.9	28.1 ±1.9	40.0 ±2.1[a]
Length (mm)	22.39	24.09[a]	22.38	25.51[a]	21.08	23.12[a]	21.95	24.24[a]
	Length standard error = ± 0.45mm							
Weight (mg)	48.0	65.7[a]	52.0	77.9[a]	44.0	75.0[a]	48.0	72.8[a]
	Weight standard error = ± 4.8mg							

[a] Indicates the value is significantly different (P<0.05) from the seawater control (SWC) at that location.

Prawn PL stocked into groundwater treatments grew significantly longer than their SWC's (Table 7.2). The average length of the PL at the start of the trial was 13.35 ± 0.45mm. After 30 days, the longest prawns, 25.51 ± 0.45mm, were present in the SE2 TGW sample. The smallest prawns, with an average length of 21.08 ± 0.45mm, were obtained from the high salinity SWC (29ppt).

The PL held in TGW were significantly heavier at day 30 than Pl held in the SWC's. (Table 7.2). The heaviest prawns, 77.90 ± 4.80mg, were observed in the SE2 TGW treatment and the lightest 44.00 ± 4.80mg, in LV1 SWC treatment.

7.4 Discussion

The results from this trial provide further evidence that once corrected for potassium deficiencies, groundwater from inland regions of Queensland can provide a favourable medium for the growth of black tiger prawns (*P. monodon*).

Although the results generated provided effective comparisons between seawater and treated groundwater, the rate of growth and survival of PL in all experimental units was generally poor compared to commercial pond data. This result can be explained by the experimental set up, specifically, the use of small white fibreglass tanks that lacked suitable substrate and access to micro-algae, zooplankton and detritus. Cawthorne et al., (1983) observed that the lack of suitable substrate may significantly influence survival of *P. monodon* PL given similar trials by Pantastico and Oliveros (1980) yielded better results for survival in freshwater where treatments had access to natural substrate. Normally at this age (18 days) PL are stocked into ponds that have been previously fertilised with organic and inorganic fertilisers to promote a favourable planktonic bloom. By rearing PL in a clear water system, PL are not provided with the array of natural food items normally found in ponds. As a result, growth at this important stage of development was compromised as it relied solely on the performance of artificial diets developed for use in pond environments.

In this study, PL survival was significantly higher in saline groundwater than in diluted seawater. As discussed previously (section 6.4), this higher average survival may be due to relatively higher concentrations of calcium in groundwater compared to seawater diluted to the same salinity. Again, previous studies have identified that marine crustaceans require relatively large amounts of calcium to complete the moulting cycle (Boyd and Thunjai, 2003). Abnormalities during moulting have been observed in freshwater crustaceans if calcium levels are under 30mg/L (Morrisey, 1970; Fieber and Lutz 1982). Freshwater crustaceans, such as redclaw (*Cherax quadricarinatus*), mineralise calcium from the old shell prior to moulting and store it in paired hemispherical stones called gastroliths (Huner and Barr, 1991). Located near the stomach these stones are dissolved into the lumen during moulting enabling the calcium to be taken up with the new shell. However, despite this ability, these crustaceans still need to absorb up to 70% of the calcium they need from the environment to fully harden their shells. Marine crustaceans do not posses gastroliths and must obtain all the calcium they need directly from the water, soil and food items.

The calcium concentration in SWC's for DD1 and SE2 approximated 21mg/L and 27mg/L respectively. These levels are below those recommended for adequate moulting in freshwater crustaceans and are likely to affected survival in this study. Calcium deficiencies would explain poor growth (final weight and length) in DD1 and SE2 SWC's relative to their TGW treatments. Additional studies are necessary to better understand the effects of calcium deficiency on prawn growth and the impact of specific water chemistries on the culture of marine prawns at low salinities.

As discussed in previous chapters, it is generally accepted that like other penaeid prawns, *P. monodon* can adapt to a range of salinities. The optimal range for growth is often recommended to lie between brackish and full strength salinities of 10 to 35ppt (Rajyalakshmi, 1980; Chen, 1984; Liao and Murai, 1986; Chanratchakool et al., 1994). In contrast Zhang et al., (1989) and Navas and Sebastian (1989), concluded that growth of *P. monodon* is optimal at salinities above 3 and 4ppt respectively. Other studies have demonstrated that this species grows favourably at 2ppt (Saha et al., 1999; Athithan et al., 2001) and even 0ppt (Pantastico and Oliveros, 1980). Saha et al., (1999) observed that growth, survival and therefore yield was better in ponds with a lower average salinity (7 to 0.16ppt) than in ponds with higher average salinity (19 to 4.6ppt) in inland production trials. The results of this study also demonstrate that *P. monodon* grows at equivalent rates at low (1.8 – 2.8ppt) and high (29ppt) salinities.

While the success of inland prawn culture in other countries bodes well for the activity in Queensland, small-scale bioassay and growth trials would need to be applied under conditions more indicative of commercial pond culture to determine its viability. For this reason, the next phase of this study involved assessing the performance of black tiger prawns in earthen ponds using saline groundwater.

8. Inland Prawn Pond Trials

8.1 Objectives

Pilot scale pond trials were undertaken to investigate the performance of *P. monodon* in earthen ponds filled with low salinity groundwater and operated using zero water discharge principles.

8.2 Methods

8.2.1 Site Selection

The pond trial site, a redclaw farm located in the Tiaro shire approximately two hours North of Brisbane, was selected because of its favourable local climate, the quality and availability of its groundwater supply, the existing infrastructure and its status as an operational aquaculture facility. Groundwater sampled from this site had been included in the previous investigations (SE1) (Chapters 3, 5, 6 and 7). Site soil analysis indicated high clay content (>70%) suitable for earthen pond construction and water holding characteristics.

8.2.2 Systems Design and Preparation

For the trial, four square-shaped earthen ponds (average size of 330m^2 each), a 300m^2 treatment reservoir, and a 400m^2 buffer storage were constructed (Figure 8.1). The ponds were bird netted to prevent predation and a 400mm barrier fence erected to prevent redclaw from neighbouring ponds from colonising the system (Figure 8.2).

Groundwater drawn from a depth of 10m was pumped to the buffer storage, where it was de-gassed and balanced with the addition of potash (KCl) prior to stocking. The treatment reservoir was used to recycle effluent water back to the production ponds while providing a store for discharge waters during harvest (Figure 8.3). A 1.5-hp propeller-aspirator provided aeration and circulation in each of the ponds, the treatment reservoir, and the buffer storage. The treatment and production ponds had an average depth of 1.5m, while the buffer storage had an average depth of 3m when full.

8.2.3 Acclimation and Stocking

8.2.3.1 2002/03 Season

Two separate acclimation and stocking events were carried out with ponds (P1 and P2) stocked first, and the remaining two ponds (P3 and P4) stocked two weeks later with a new batch of PL.

P. monodon PL_{15} were held in aerated 10m^3 tanks at the Bribe Island Aquaculture Research Centre for 7 days. Here the salinity was lowered from 34pppt to 10ppt using dechlorinated municipal water. All animals were then transported to the Tiaro site. On arrival the salinity was lowered further to 4,670µS/cm (2.9 ppt) using pond water in 2m^3 tanks at a rate of 0.19ppt hr^{-1} (Figure 8.4). The PL were fed both a commercial prawn diet as well as live artemia nauplii during both the holding and acclimation periods.

Once the target salinity was reached, PL were counted and stocked at 22-25/m^2. During each of the two stocking events, a number of PL were also stocked into floating hapas nets to monitor survival and vigour for two weeks post-stocking (Figure 8.5).

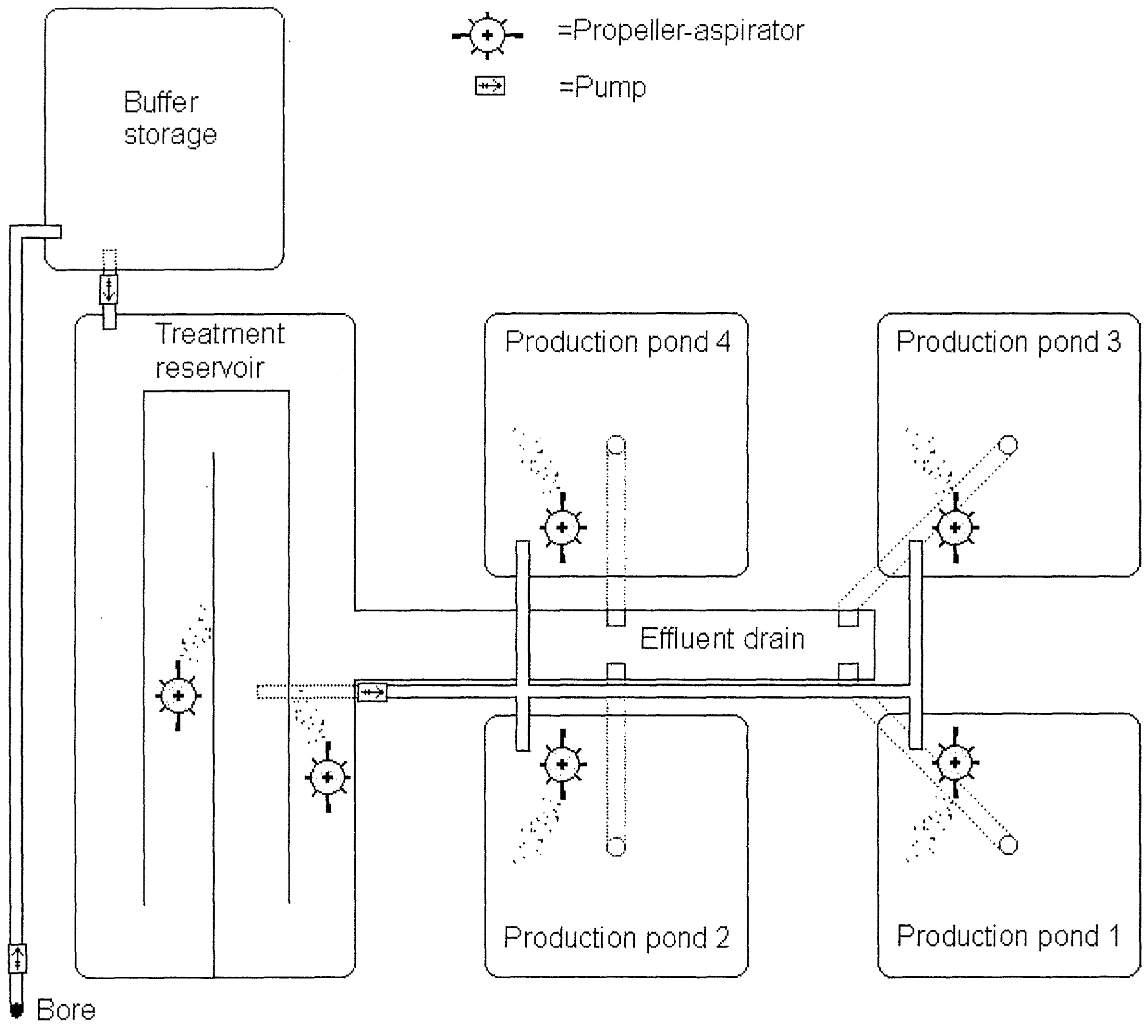


Figure 8.1. Schematic representation of the pond system established at Tiaro in southern Queensland. Four square-shaped earthen ponds (average size of 330m^2 each), a 300m^2 treatment reservoir, and a 400m^2 buffer storage were constructed to recirculate but not discharge water during the cropping cycle.

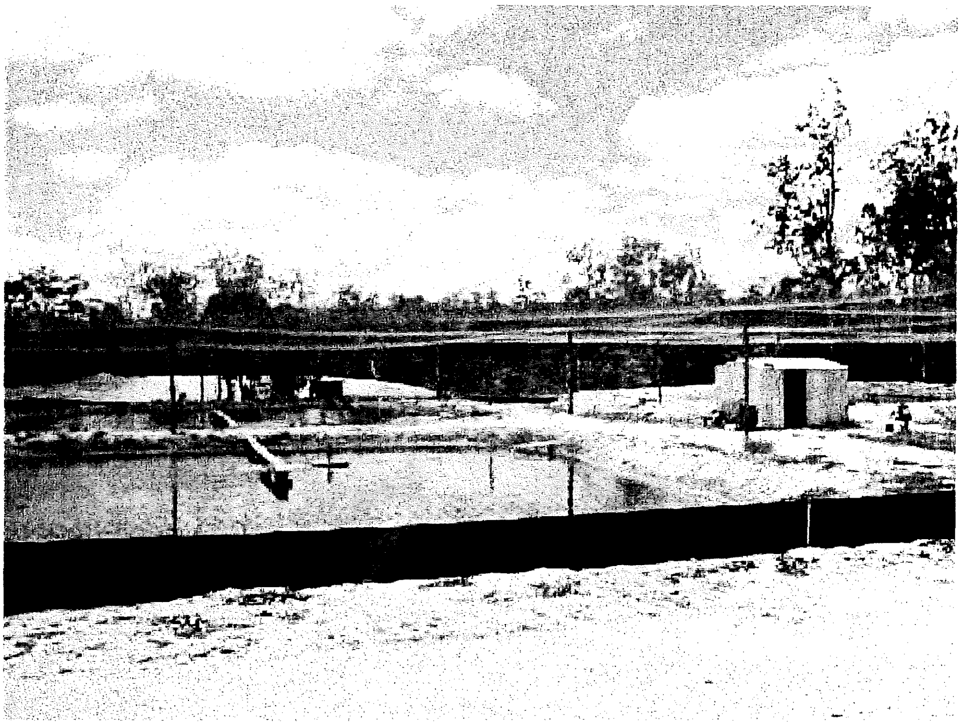


Figure 8.2. Trial ponds were covered with bird netting to reduce bird predation while a predator fence was erected to prevent movement of redclaw into the system from neighbouring ponds.

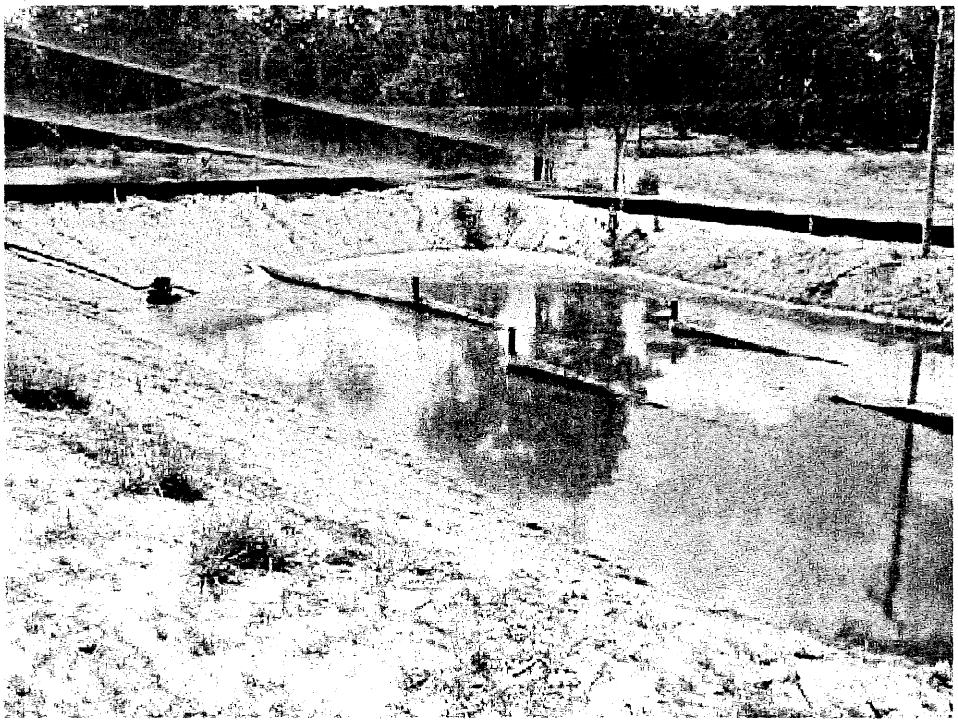


Figure 8.3. The 300m^2 treatment pond was partitioned to encourage directional water flow to maximize solids settlement and treatment of wastes.

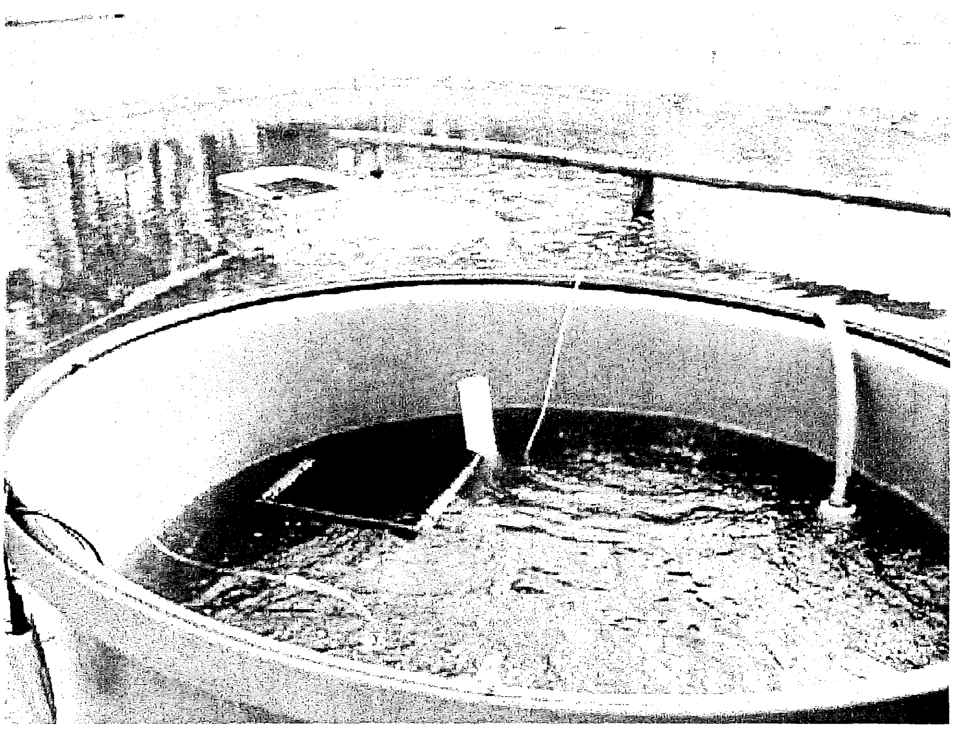


Figure 8.4. Acclimation tanks ($2m^3$) were used on-site to lower the salinity from 10 to 2.9ppt at a rate of 0.19ppt hr^{-1}. A hapas net used to monitor survival and vigour post-stocking can be seen adjacent to the pond walkway.

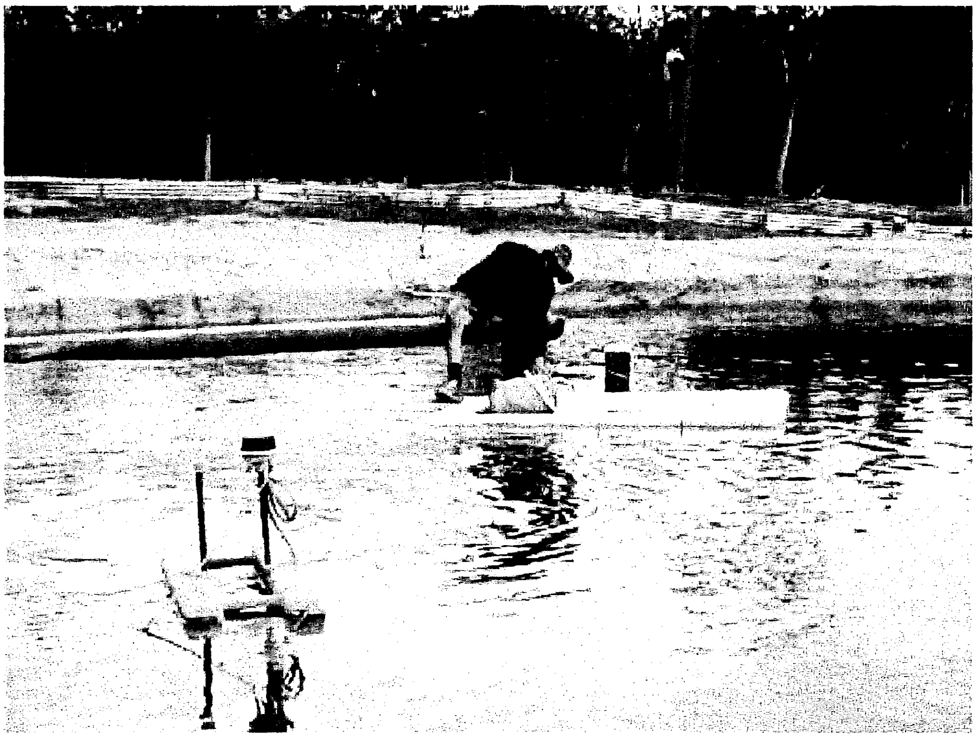


Figure 8.5. Hapas nets were stocked with PL to monitor survival and vigour for 2 weeks post-stocking.

8.2.3.2 2003/04 Season

As in the previous season, acclimation and stocking of the four ponds was achieved in two separate events, with two ponds (P3 and P4) stocked first and the remaining ponds (P1 and P2) stocked 9 days later with a new batch of PL.

The acclimation of the first batch of prawns was again undertaken at BIARC using a similar methodology to that described for the 2002/03 season. The difference was that the acclimation commenced immediately upon the receipt of PL from the hatchery. In contrast, the second batch of PL, sourced from the same hatchery, were acclimated on-site using the site's groundwater.

Stocking of the first batch of PL involved hand counting while decanting them into P3 and P4. These ponds were stocked at a rate of 31.6 and 32 PL/m^2 respectively. Approximately 40,000 animals made up the second batch of PL to arrive from the hatchery. These animals were not counted following their acclimation but volumetrically divided and equally distributed into P1 and P2 to give approximately 57.5 and 48.9 PL/m^2 respectively.

Hapa nets were not used to assess the prawn's survival following acclimation in this season.

8.2.4 Husbandry and Feeding

A commercial feed (Charoen Pokphand) was provided to the prawns four times per day (approximately 06:00hrs, 10:00hrs, 14:00hrs, 18:00hrs) at a rate prescribed by the feed's manufacturer. In the 2002/03 season, daily checks of feed trays were conducted in each pond. These observations were used to adjust the quantity of the feed prescribed to meet the prawn's immediate appetite and compensate for any mortality.

In 2003/04 feed trays were not used for the purpose of adjusting feeding rates. Instead feed demand was estimated through an extrapolation of the prawn's weight obtained from sub-sampling and estimated survival.

Daily checks of prawn health were conducted using feed trays or less regularly by cast net. Visual inspections were conducted to assess the physical condition of the carapace, antennae and gills. Pond edges were routinely inspected for moribund prawns.

In both years weekly weight checks were also conducted using animals collected using feed trays and fine meshed dip nets until the animals grew to a size of approximately 5g (Figure 8.6). After this point, a cast net was used to collect samples. Typically, fifty (50) animals were sampled from each pond, counted, and their pooled weight recorded before being returned to the pond.

8.2.5 Water Quality and Pond Management

Using a multi-probe water quality meter, temperature, pH, dissolved oxygen and conductivity were measured in each pond twice daily. Secchi depth was also recorded with the same frequency.

Ammonia-N and Nitrite-N were monitored weekly through the season using a transmittance-display photometer. Groundwater and pond water samples were provided for laboratory analysis of ionic composition to investigate temporal changes in ion balance.

Additional water via the buffer pond was added to the production system during the season to compensate for evaporative loss as required. The relatively small volumes

Figure 8.6. These juvenile prawns were collected for the purpose of weekly weight checks. Animals were collected using feed trays and fine meshed dip nets until approximately 5g after which cast nets were used for sub-sampling.

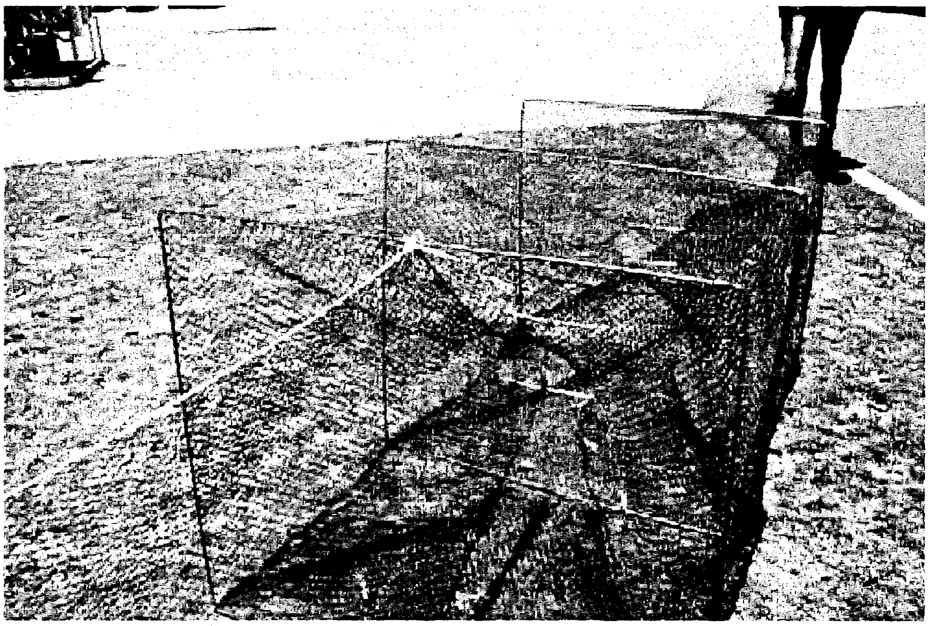


Figure 8.7. A fyke net used to harvest prawns. The nets are set along the edges of the pond and take advantage of the behaviour of *Penaeus monodon* to move against the current.

required to balance this evaporative loss did not require the further addition of KCl to the system.

A mid-crop dosage of agricultural lime at a rate equivalent to 1,000kg/ha and daily additions of molasses at 4 to 8mg/L were applied to help flocculate suspended material and minimize diurnal fluctuations in pH.

The propeller-aspirators were positioned to provide maximum circulation and assist the settlement of solids in and around the centre of the pond. On a weekly basis the external standpipes were removed to dump solids which had settled within the pond's outlet pipe. Additionally, the internal screened standpipes were scrubbed weekly to remove algae and improve water flow.

8.2.6 Harvest

In 2002/03 the prawns were drain harvested through a harvest basket attached to the pond's outlet. During this process the prawns would be removed from the harvest bag, washed, batch weighed, graded and then placed on ice until cooking. Randomly selected samples of 100 prawns from each pond were individually weighed. This information was used to calculated mean harvest weight, survival and food conversion ratio (FCR).

In 2003/04 the same drain harvest method was used to harvest P1 and P2. However, part of the crop from P3 and P4 was harvested over a period of several weeks using fyke nets (Figure 8.7). This method of harvesting was employed to enable smaller quantities of fresh prawns to be provided to seafood traders as required. The remaining prawns in P3 and P4 were then drain harvested.

<u>8.3 Results</u>

8.3.1 Production

8.3.1.1 2002/03 Season

All four ponds were harvested at the same time, representing a total growout period of 103 and 92 days for the two stocking events (Table 8.1). Prawns reached an average size of 20.4g and an average survival of 56 to 78%. Mortality in hapa nets stocked with PL was up to 25% two weeks after stocking. Animals from ponds P3 and P4 grew faster (average weekly growth of 1.6 g/week) than those from P1 and P2 (1.3 g/week). These results represent an equivalent average yield of 2.97 t/ha/crop for the four ponds. No signs of parasitic infection or disease were observed.

8.3.1.2 2003/04 Season

Above average growth in P2 (Table 8.2) enabled it to be harvested 84 days after stocking (Figure 8.8). Net harvest of P4 was conducted over a period of several weeks until day 108. After this time the remaining 10.4kg of stock was collected by drain harvest. In this case fyke netting removed approximately 92% of the stock. Approximately half of P3 was cropped using the same method. Unfortunately, an overnight power failure resulted in complete mortality for prawns remaining in P3 as well as the entire stock in P1. This event resulted a production period of 107 and 118 days respectively for P1 and P3. Two ponds, P1 and P2, were stocked at the relatively high densities of 48.9 and 57.5 PL/m^2, compared to the first batch of PL that were stocked into P3 and P4 at 31.6 to 32 PL/m^2. Despite these disparate stocking rates, differences in survival rates resulted in similar overall production rates across all ponds that averaged 4.22 t/ha. The average growth rate was 1.8 g per week.

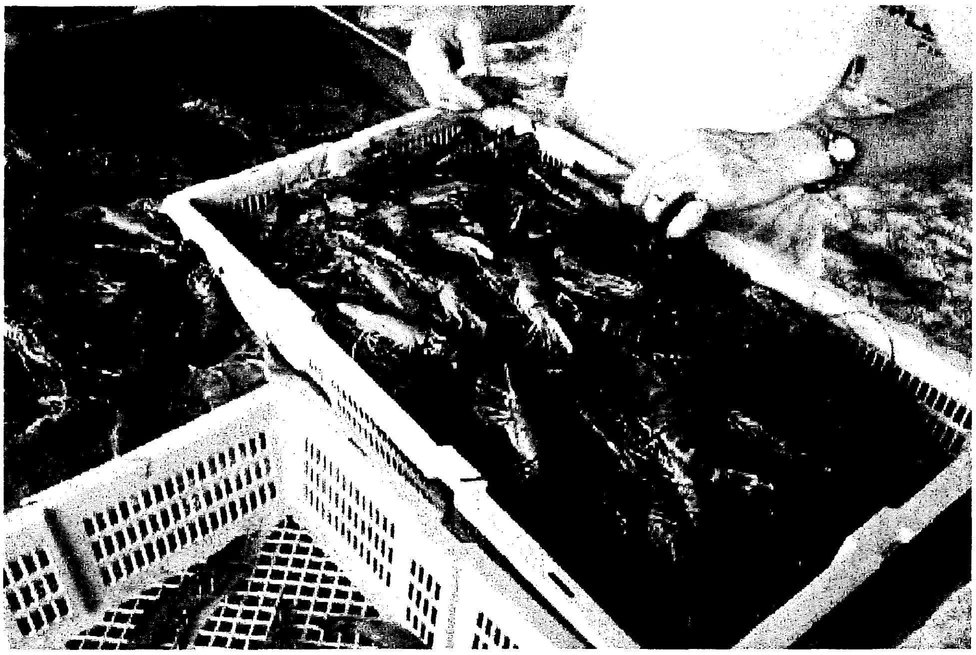


Figure 8.8. Black tiger prawns (*Penaeus monodon*) being sorted following harvest from Pond 1 during the 2003/04 production trials.

Figure 8.9. Cooked prawns from the 2002/03 harvest of inland farming trials at Tiaro. This product was received well by both buyers and consumers both in terms of its appearance and taste.

Table 8.1. 2002/03 Production cycle and harvest summary.

Pond	P1	P2	P3	P4	Average
Surface area (m^2)	313	368	316	320	**330**
Growout time (days)	103	103	92	92	**97.5**
Stocking density (PL/m^2)	25.5	21.7	22.1	21.8	**22.8**
Average stocked weight (g)	0.03	0.03	0.03	0.03	**0.03**
Average harvest weight (g)	19.4	19.1	21.5	21.4	**20.4**
Average growth/week (g)	1.3	1.2	1.6	1.6	**1.4**
Production rate (t/ha)	3.88	2.33	2.81	2.85	**2.97**
Production yield ($No./m^2$)	19.9	12.2	13	13.3	**14.4**
Survival (%)	78	56	59	61	**63**
FCR	1.49	2.17	1.99	1.92	**1.89**

Table 8.2. 2003/04 Production cycle and harvest summary.

Pond	P1	P2	P3	P4	Average
Surface area (m^2)	313	368	316	320	**330**
Growout time (days)	107	84	118	108	**104.3**
Stocking density (PL/m^2)	57.5	48.9	31.6	32	**42.4**
Average stocked weight (g)	0.0058	0.0058	0.0058	0.0058	**0.0058**
Average harvest weight (g)	26.3	27.7	24.5	25.3	**26.5**
Average growth/week (g)	1.7	2.3	1.5	1.6	**1.8**
Production rate (t/ha)	4.02	3.85	4.62	4.38	**4.22**
Production yield ($No./m^2$)	16.1	12.2	19.6	18.2	**16.1**
Survival (%)	28	25	62	57	**38**
FCR	3.36	2.18	2.72	2.38	**2.66**

8.3.2 Market Feedback

The prawns were sold directly to several restaurants and seafood traders (Figure 8.9). Feedback concerning the product's quality was excellent and highlighted the sweet taste, firm texture and brilliant colour of the prawns.

8.3.3 Water Chemistry

The chemical composition of the groundwater supplied to the buffer storage is presented in Table 8.3. The balance of the major ions remained stable throughout the trial period. The addition of KCl raised potassium levels from 5.9mg/L to 40mg/L.

Table 8.3. Chemical composition of Tiaro groundwater before treatment with potassium chloride.

Parameter	Value
Conductivity @ 25°C	4,670 µS/L
Total hardness as $CaCO_3$	461 mg/L
Alkalinity as $CaCO_3$	130 mg/L
Calcium – Filtered	60 mg/L
Magnesium – Filtered	75.3 mg/L
Sodium – Filtered	888 mg/L
Potassium – Filtered	5.9 mg/L

8.3.4 Water quality

8.3.4.1 2002/03 Season

During this trial pond temperatures averaged 27.6°C and ranged between 33.1°C and 20.4°C (Table 8.4). Pond pH averaged 8.1 but spiked to 10 during the early part of the season. Conductivity averaged 4,531 µS/mg and fluctuated slightly due to evaporation and rainfall events. Dissolved oxygen (DO) levels fell no further than 4.8 mg/L and averaged 7 mg/L over the season. Secchi depth varied greatly from 5cm to 80cm during the trial and averaged 45cm. Ammonia levels peaked towards the end of the crop reaching 1.15mg/L while nitrite levels were highest mid crop at 0.59mg/L. The average un-ionised ammonia and nitrite levels were 0.16 and 0.13 mg/L respectively.

8.3.4.2 2003/04 Season

The temperature profile was very similar to the 2002/03 season, averaging 27.5°C and ranging between 33.5°C and 21.2°C (Table 8.4). Similarly, PH averaged 7.8 however its range was slightly more constricted, falling between 9.1 and 6.9. The average conductivity was slightly higher at 4,878 µS/mg as was mean DO at 7.5 mg/L. Again, secchi ranged broadly and averaged 39cm. As in 2002/03 ammonia levels were highest toward the end of the crop but at 0.59 mg/L were less than half of the peak values observed in 2002/03, despite higher yields. Ammonia levels were however generally low with an average of 0.17 mg/L. Nitrite levels were also highest at the end of the crop reaching 0.23 mg/L but were generally much lower than the previous year with an average of 0.07 mg/L.

Table 8.4. Maximum, minimum and average values for various water quality indicators in production ponds for the 2002/03 and 2003/04 seasons.

Parameter	Season	Max	Min	Average
Temp (°C)	2002/03	33.1	20.4	**27.6 ± 0.08**
	2003/04	33.5	21.2	**27.5 ± 0.13**
PH	2002/03	10.0	6.9	**8.1 ± 0.02**
	2003/04	9.1	6.9	**7.8 ± 0.01**
Conductivity (µS/mg) @ 25°C	2002/03	5,000	3,437	**4,531 ± 12**
	2003/04	5,781	3,437	**4,878 ± 28**
DO (mg/L)	2002/03	10.6	4.8	**7.0 ± 0.04**
	2003/04	10.2	2.9	**7.5 ± 0.06**
Secchi (cm)	2002/03	80	5	**45 ± 0.60**
	2003/04	80	10	**39 ± 1.51**
Un-ionised ammonia (mg/L)	2002/03	1.15	<0.01	**0.16 ± 0.03**
	2003/04	0.59	<0.01	**0.17 ± 0.02**
Nitrite (mg/L)	2002/03	0.59	<0.01	**0.13 ± 0.02**
	2003/04	0.23	<0.01	**0.07 ± 0.02**

8.4 Discussion

The results of these trials demonstrate that inland production of marine prawns in Queensland is viable and can deliver good yields with the potential to develop a range of production systems including 'zero-discharge' farms. The growth rates achieved in these trials of 1.4 and 1.8g/week, are comparable with those recommended for commercial production of this species in Queensland (QDPI, 2000). The improved growth rate and yield in the second year probably reflected the stabilization of the ponds and the system itself as well as some refinement of management protocols and operator experience.

The average pond yields of 2.97 and 4.22 t/ha/crop were commensurate with the average pond yields for Queensland coastal prawn farms of between 3,406 and 4,045tkg/ha/crop in recent years (Lobegeiger, 2004). However, direct comparisons of yields between the small ponds used in this study (330m^2) and those used by industry (1ha) may not be truly representative of the trial system's performance. The comparative loss of productive pond area in smaller ponds may have effectively resulted in an over-estimation of the pond's productive area. The gentle slope of the pond walls (2:1), consumed a relatively high proportion of the trial pond's total surface area (approximately 15%). As prawns did not utilize the shallow, visible areas of the pond bank during the day, this area is lost to feeding. As a consequence, feed was typically distributed at least 2m or more out from the pond's edge resulting in a loss of productive area. In a 1ha pond, similarly sloping walls would represent a comparatively smaller proportion of the total surface area (<4%).

This comparative loss in surface area may have resulted in an underestimation of equivalent actual yield in larger ponds.

Another factor making direct comparison of small and large ponds difficult is the relative spread of organic wastes on the bottom of ponds. The accumulation of organic matter (uneaten food, faeces, dead algae, zooplankton etc) and clay sediment in the centre of the pond was comparatively high in the trial ponds (almost 30% of pond bottom surface area) compared to larger ponds that can be as low as 18% (Avnimelech and Ritvo, 2001). Organic matter or 'sludge' leads to the development of anaerobic sediments whose presence can limit growth and reduce survival. Feeding activity in these areas is less than in sludge free areas. Prawn capture rates in the middle of ponds, where deposited sediments are greatest, are less than half those near the ponds edges where substrate conditions are at their best (Avnimelech and Ritvo, 2001). It would be expected that in larger, well managed ponds, the relative increase in productive area (of up to 20%) would make the yields achieved in this trial comparatively easier to obtain.

Average survival rates varied between ponds and seasons. This variation appears to be influenced by a combination of stocking density, stocking protocols and the productive capacity of the ponds themselves. An average survival of 63% was observed in 2002/03 when the mean stocking rate was less than 23 PL/m^2. In 2003/04 the average survival was low by comparison at just 38%. This low average survival is primarily the result of poor survival in ponds 1 and 2 (25 and 28%). Survival in ponds 1 and 2 was less than half the survival observed in ponds 3 and 4 (65 and 58%). Ponds 1 and 2 were stocked at much higher rates of 57.5 and 48.9 PL/m^2 respectively compared to ponds 3 and 4 which were stocked at 31.6 and 32 PL/m^2 each. The high stocking rates in ponds 1 and 2 may have exceeded their productive capacity given the final yields from all ponds were similar. If this were correct, the ponds in their current configuration would have a productive capacity of just under 5t/ha/crop regardless of stocking rate or management input. However, these ponds were stocked at different times using slightly different protocols. The PL for ponds 1 and 2 were acclimated on-site, direct from the hatchery, using the site's groundwater. This was different to the procedure used for ponds 3 and 4 that were partially acclimated before reaching the site. No hapa nets were used to determine post-acclimation survival during these later trials. In the absence of this data, the efficacy of the acclimation method used for ponds 1 and 2 and its impact on survival and pond yield cannot be determined. Other management practices such as the use of partial harvesting methods (Chamberlain et al., 2002), may enable the production season to be extended and the total pond yield increased above that obtained in this study.

The average temperature, pH and dissolved oxygen (DO) levels observed in both trials fell within recommended parameters for the production of black tiger prawns. The best growth for this species is observed when water temperatures are stable and above 25°C. Water temperatures in this study averaged 27.5°C and did not vary more than one degree diurnally. Aeration levels relative to production was high in this study as were the average DO levels of 7.0mg/L in 2002/03 and 7.5mg/L in 2003/04. While DO levels did reach as low as 4.5 and 2.9 mg/L for a few days towards the end of their respective seasons these levels were not typical. In 2003/04 a power failure resulted in complete mortality of the remaining stock when DO levels were at their lowest. At this time the secchi depth in ponds was also low as the result of a strong algal bloom. While *P. monodon* can tolerate short periods of DO less than 2mg/L, studies have shown that DO concentrations in prawn ponds should not decline below 50% of saturation (McGraw et al., 2001). The typically high DO levels observed in this study were clearly sufficient for good growth.

Under normal semi-intensive conditions 1kw of aeration is required to produce 1t of prawns using conventional paddlewheel arrangements (QDPI, 2002). In this study each pond was fitted with a single 1.5-hp propeller-aspirator. In a large pond this would support 1t of production but in this trial was used to provide aeration for a maximum of just 152kg. More efficient stocking and aeration strategies will be investigated in future trials.

While feed conversion efficiencies were variable in these trials, the elevated FCR values observed in the 2003/04 season demonstrates the importance of monitoring feed inputs. In the first season, 2002/03, feed trays were used in combination with corrected stocking rates from survival estimates obtained from the hapa nets, to determine feed volumes and frequency. In the second year neither feed trays or hapa nets were used to guide feed inputs. The resulting FCR values in all ponds were comparatively high with an average of 2.66:1. In a closed system, additional wastes generated by overfeeding can result in a serious deterioration in water quality, especially during the later stages of the crop. As organic wastes decay nitrogen 're-mineralises' to its inorganic forms, ammonia and nitrite, which are rapidly utilized by blue-green algae. It is highly recommended that feed consumption be actively monitored through the use of feed trays to prevent overfeeding. This issue will be addressed in future trials as efforts are made to increase production from the system.

One of the major factors limiting prawn production in open ponds is the accumulation of inorganic nitrogen (ammonia and nitrite), which can reduce prawn growth or even result in death at high concentrations. Prawns excrete ammonia from the gills, in urine and faeces. It can also be released from decaying organic matter. It is present in two forms, un-ionised ammonia (NH_3), which is toxic and ammonium (NH_4^+), which is relatively harmless. At neutral pH all ammonia is present in the NH_4^+ form but this equilibrium shifts with an increase in pH. When above pH 9, half of the NH_4^+ is converted to NH_3. The 96hr lethal dose of NH_3 for *P. monodon* is 1.26 mg/L (Chin and Chen, 1987). The average ammonia concentrations observed in this study of 0.16 and 0.17mg/L in 2002/03 and 2003/04 are close to the safe limit proposed for pond production by Chen and Chin (1987) of 0.13mg/L. However, given the relative toxicity of ammonia can quickly increase with pH and higher temperatures, it is wise not to exceed these recommendations.

Ammonia is converted by bacteria to relatively harmless nitrate, however the intermediate product of this reaction, nitrite, is toxic to crustaceans. Like ammonia this toxicity changes with pH and salinity. Lin and Chin (2003) demonstrated that as salinity decreases from 35ppt to 15ppt nitrite toxicity increases by 421%. This occurs because in higher salinity water the presence of chloride ions prevents the uptake of nitrite. Safe nitrite concentrations for *P. monodon* PL in marine ponds have been estimated at around 1.36mg/L (Chen and Chin, 1988a). However, this figure will not necessarily be 'safe' in low salinity environments and care should be taken to keep levels well under 0.5mg/L. Water quality management in intensive 'low salinity' zero-discharge systems will be particularly critical and should be the subject of further studies in the future.

In both seasons, the pH of water in production ponds was relatively stable. The exception was in the 2002/03 period where pH levels peaked at 10 almost one month into the trial. After this point molasses was added at a rate of 4 to 8 mg/L per day. The effect of these additions was a reduction and stabilization of pH values. The addition of carbon to the pond promotes bacterial production which acts to help assimilate ammonia, reduce its toxicity, and if properly managed, remove the problem of inorganic nitrogen accumulation altogether (Avnimelech, 1999). By adding molasses in the trial ponds, bacterial growth was stimulated although no attempt was made to create a fully heterotrophic system. The bacterial flocs that form in heterotrophic systems have been shown to act as a secondary food for *P. vannamei* (Chamberlain et al., 2001). No studies have demonstrated its value to *P. monodon*. Other species such as *P. merguiensis* may be able to utilize bacterial flocs in a similar fashion to *P. vannamei* given they have similar feeding behaviours.

More intensive, heterotrophic systems will be investigated in future trials using lined zero-exchange and recirculated ponds.

As mentioned previously, saline water is present in inland regions in both shallow and deep aquifers, is associated with many grazing, irrigation, mining and desalinisation industries, and is characteristic of many ephemeral inland water bodies. Yet as discussed, not all of these various sources of saline water will be suitable for prawn farming, while issues of climate and infrastructure may also limit opportunities in some regions. Where favourable prawn farming conditions occur, the long-term impact of this activity on the surrounding environment, including underlying freshwater aquifers, surface waters and soils, must be considered.

Concerns over the salinisation of freshwater rice growing areas are what prompted the Thai government to place a temporary ban on inland *P. monodon* production in 1998 (Szuster and Flaherty, 2000). While it is estimated that at its peak the inland prawn industry in Thailand covered some 22,000ha, the impact from much of this was poorly managed. While some farmers used saline groundwater for this activity, most introduced brine from coastal salt farms to elevate salinity. The discharge from these farms made its way into local freshwater rivers and irrigation schemes, degrading the environment and endangering downstream rice production. The development of inland prawn farming in Queensland must not contribute to the salinisation of the surrounding landscape. Appropriate farm design, management and regulation must be adopted to ensure sustainable development of this activity.

The recent salinity hazard maps generated by the Queensland Department of Natural Resources and Mines (DNRM) indicate that some 4.2 million hectares of land in the Queensland Murray-Darling Basin (QMDB) has a high potential to develop problems with salinity (DNRM, 2002). The QMDB constitutes 15% of the total area of the Murray-Darling basin. Similarly, rising salinity levels are the biggest single environmental issue facing farmers in the Burdekin River delta. Any new farming system in these areas must not contribute to the problems of salinity in these regions.

Boyd (2001) noted that inland prawn farming can be conducted without causing adverse environmental effects provided a number of provisions concerning site development and management were observed. These include the prevention of discharge waters (effluent or as run-off); proper pond construction to prevent lateral and horizontal seepage (including impermeable pond liners if needed); recycling and re-use of culture waters; containment and management of solid wastes; and installation of monitoring infrastructure (including vegetative barriers and piezometers). In this study no water was released from the farm. Pond sediments removed between seasons was used as topsoil to revegetate un-grassed areas adjacent to the ponds and vegetation near the trial ponds was monitored for signs of salt scalding.

Although the production technologies used in this trial were limited, they demonstrate the potential for low salinity inland prawn farming in Queensland. Higher relative yields may be obtained from larger ponds while the incorporation of pond liners to reduce turbidity and increase flow rates may make pond management easier and assist overall production efficiencies. Highly intensive systems have proven successful with *P. vannamei* overseas. These trials were conducted at semi-intensive levels in earthen ponds as is common for inland production of *P. monodon*. The efficacy of highly intensive systems for *P. monodon* production must be further investigated before the comparatively large investment required for their establishment and operation can be justified.

9. General Discussion

The objectives of these investigations were to review data concerning groundwater use, suitability and availability in key regions; establish methods to permit the rapid acclimation and transfer of marine prawns to fresh and low salinity groundwater for growout; ascertain the mineral supplementation required to enable individual groundwater sources to be used for prawn culture; and compare the growth of black tiger prawns in marine and inland saline waters. The findings of these investigations can be summarised as follows:

- The comparative abundance of fresh, low salinity, brackish and saline water suitable for prawn culture in several regions in Queensland is high.
- Of the 8,500 bore records studied 81% have conductivities recognised as being suitable for black tiger prawn (*P. monodon*) production (800µS/cm or greater).
- 33% of all bores studied have conductivities in excess of 3,000µS/cm.
- Regions like the Burdekin delta and the Darling Downs generally posses good quality groundwater with chemistries favourable for the production of marine prawns.
- A simple analysis of groundwater chemistry does not necessarily provide all of the necessary information to determine its suitability for prawn culture.
- Acclimation of prawn postlarvae to low salinity environments should be conducted slowly and with minimal disturbance so as to maximise survival.
- Acclimation procedures and target salinities will be species and salinity dependent with *P. monodon* being more tolerant of lower salinities than *P. merguiensis*.
- Some groundwater types are more suitable than others but most are deficient in potassium (K^+).
- In most cases, the addition of K^+ chloride (KCl) will improve survival significantly in groundwater deficient in K^+.
- Additional studies are required on the impacts of groundwater chemistry (e.g. calcium), particularly at low salinities, on the culture of marine prawns.
- Laboratory trials at salinities greater than 2,800µS/cm show that acclimated *P. monodon* postlarvae will grow equally well in groundwater regardless of salinity at salinities as low as 3,000µS/cm.
- Pond trials show that *P. monodon* grow at favourable rates in low salinity groundwater (>3,870µS/cm) under semi-intensive conditions.
- Fully recirculated semi-intensive farming is technically feasible using low salinity groundwater.
- The efficacy of highly intensive systems for *P. monodon* production must be further investigated before the comparatively large investment required for their establishment and operation can be justified for this species.
- Further research on closed 'zero-discharge' and 'zero-exchange' systems is required for *P. monodon* at a range of intensification levels.

This series of investigations demonstrate that inland production of marine prawns using groundwater in Queensland could be undertaken in a number of regions using available infrastructure and groundwater resources. The ability to exploit this opportunity will be largely contingent on industry's adherence to principles of sustainable development and management. The level of intensity, investment and risk will be highly dependent on the location in which the activity is being conducted, its water supply, chemistry, and very importantly the skill of the operator.

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